Russian River Human Impact Study
PhyloChip Microbial Community Analysis

Final Report
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I. Executive Summary

Background and Approach

This project focused on microbiological source identification in the middle and lower Russian River watershed. Goals of this study included collection of the principal data needs required to understand sources of pathogenic indicator organisms and understand microbiological transport mechanisms.

Monitoring tasks were identified for the following five management questions:

1. What is the spatial variability of the microbial community in the Russian River?
2. What is the temporal variability of the microbial community in the Russian River?
3. Do land uses influence the variability of the microbial community in the Russian River watershed?
4. Do recreational beach areas influence the variability of the microbial community?
5. Do areas with onsite water treatment influence the variability of the microbial community in the Russian River watershed?

A new technology is available that can greatly improve microbial source identification. PhyloChip DNA microarray contains 1.1 million probes that capture representatives of all known, nearly complete 16S rRNA genes in public databases. The PhyloChip can quantify over 59,000 bacterial taxa in a single sample by targeting variations in the 16S rRNA gene. The 16S rRNA gene is universally present in all microbes and small sequence variations within the gene can be used as a “barcode” for bacteria and archaea identification. The analysis quantifies changes in relative abundance of each gene sequence and corresponding bacterial taxa among samples in the study. Recent studies demonstrate the usefulness and performance of this technology for microbial source tracking (Dubinsky et al. 2012, Cao et al. 2013).

To support the development of the Russian River Pathogen TMDL, LBNL used PhyloChip to analyze filters of water quality samples that were collected by the North Coast Regional Quality Control Board. Water sampling efforts were conducted with four (4) monitoring tasks. Task 1 was designed to assess spatial and seasonal variability of the microbial community and diagnostic fecal bacteria in the Russian River and impaired tributaries. Task 2 was designed to evaluate the influence of land use on the microbial community and diagnostic fecal bacteria. Task 3 was designed to evaluate the influence of recreational beach use on the microbial community and diagnostic fecal bacteria. Task 4 was designed to assess the influence of locations with onsite wastewater treatment systems on the microbial community and diagnostic fecal bacteria.

Summary of Results

Task 1. Site Variability

The results of the Site Variability study showed bacterial communities in the dry period were similar among all Russian River beach sites from Commisky Station Road to Monte Rio Beach and were largely composed of Betaproteobacteria, Actinobacteria and Flavobacteria that are likely native to the river. Pelagibacteria that are characteristic of marine waters were the most frequently detected taxa at Jenner in the dry period reflecting the influence of the Pacific Ocean on the mouth of the river. No fecal signal was found in any dry period samples collected from the Russian River.

In the wet period samples, the bacterial community composition was similar to dry period samples from Commisky Station Road to Memorial Beach, but diverged in composition at Steelhead Beach and was increasingly distinct at Forestville Access, Johnson's Beach and Monte Rio Beach. Diagnostic human fecal bacteria were detected at Johnson's Beach and Monte Rio Beach. Large numbers of potentially pathogenic *Staphylococcus* were detected at these sites along with human fecal bacteria. Traditional fecal indicator tests (*Enterococcus*, *E. coli*, total coliforms) did not exceed water quality limits (CDHS 2011) at Johnson's Beach and Monte Rio Beach where PhyloChip detected human fecal bacteria and *Staphylococcus*. Conversely, the bacterial community did not contain human fecal bacteria at several upstream locations where conventional fecal indicators did exceed concentration limits. Upstream sites (Commisky Station, Cloverdale River Park and Geyserville Bridge) contained *Yersinia* taxa in both wet and dry periods but there was no detected fecal source at these sites. Fecal bacteria that are diagnostic of grazing animals were detected at Steelhead, Forestville Access, Johnson's and Monte Rio Beach. More refined assessment of the grazer source was inconclusive but results suggest that cows and/or deer may contribute to the signal.

Dry period samples from tributaries in impaired watersheds contained a greater variety of taxa than the Russian River and contained increased numbers Alpha-, Beta- and Gammaproteobacteria. Dry period samples at Green Valley Creek and Santa Rosa Creek exceeded concentration limits for *Enterococcus*, but not *E. coli*, and the bacterial community did not contain diagnostic fecal bacteria. In the wet period, Green Valley Creek, Santa Rosa Creek and Laguna de Santa Rosa exceeded all fecal indicator tests but contained low numbers of diagnostic fecal bacteria.

Task 2. Land Use Variability

Results from the land use study showed no significant effects of land use on the composition or structure of bacterial communities. Taxonomic richness in all land uses was significantly greater during wet periods than dry periods for all land use types and was associated with high counts of fecal indicator bacteria. Bacterial communities converged in composition and structure during the wet period, regardless of land use type, and contained large numbers of non-fecal Bacteroidetes, and Proteobacteria that were mainly Enterobacteria (coliforms) and Pseudomonas.

Human fecal signal was not detected in dry period samples with the exception of Limerick Creek, a developed onsite septic location. Samples from developed sewered areas also had possible human fecal signal during the wet period. Developed areas with onsite septic systems generally lacked human markers in the wet period. High fecal indicator counts at these sites during the wet period were not associated with a human fecal signal.

Grazing animal signal was not found in any land use samples during the dry period but several wet period samples from different land uses contained possible fecal signal from grazing animals.

Task 3: Recreational Beach Use

In the recreational beach use study (Task 3), there was human fecal signal at Johnson's Beach in one sample at the end of the monitoring period. This sample was associated with an *Enterococcus* concentration that marginally exceeded the water quality limit (63 MPN/100mL) (CDHS 2011) but the *E. coli* concentration was below the concentration limit. There was no indication of human fecal signal in the samples analyzed from Monte Rio Beach.

Task 4: Effects of Onsite Wastewater Treatment Systems

In the onsite wastewater treatment study, there were no significant differences in bacterial communities associated with parcel density or septic risk. No sites in areas with both high parcel density and high septic risk contained evidence of human fecal signal in spite of high numbers of fecal indicator bacteria. In areas with high parcel density and low septic risk, one site (Site 5) was found to have probable human fecal signal on two sampling dates. No human fecal signal was detected at low parcel density sites with both low and high septic risk. In the three additional catchments of interest that were analyzed, site 14 had a strong human fecal signal.

There were no trends in bacterial communities associated with samples that exceeded concentration limits of *Enterococcus* fecal indicators but had low concentrations *E. coli* fecal indicators.

Conclusions

Wet periods have strong effect on the bacterial community at Russian River beaches in the lower watershed and on creeks in all land use types. The PhyloChip assay detected likely human fecal signal at Johnson's Beach and Monte Rio Beach, and indicated possible risk from pathogenic *Staphylococcus* at these locations during wet periods. Recreational beach use was also associated with human fecal signal. The inconsistency of conventional fecal indicator tests in detecting these risks warrants further investigation.

At other locations upstream in the Russian River, in impaired tributaries, and throughout the surrounding watershed, samples with exceedances in fecal indicator bacteria were frequently unassociated with fecal bacterial taxa. Similarly, many exceedances in areas with high septic risks and high numbers of fecal indicator bacteria had no fecal signal in the microbial community. These results indicate that non-fecal sources are likely supplying *Enterococcus* and coliforms to monitored waters.

The absence of significant bacterial community signatures for different land use types indicates that generalizable land use signatures may not be available for source tracking on a landscape scale. There were, however, distinct bacterial communities measured in different creeks that may be useful for tracking downstream influence. In addition, the use of microbial community analysis holds great potential to further identify potential non-fecal sources of fecal indicator bacteria that appear to be important in the Russian River watershed.

II. Project Description

Introduction

Currently, there is insufficient understanding concerning the composition of the overall microbial population (microbiome) and variations therein to accurately assess the risk to the bathing public from the presence of pathogens using the current indicator organism methodology. This lack of understanding and other issues also make it difficult to assess the effectiveness of pathogen reduction by pollution control projects.

A major problem facing the regulators is that there is lack of information regarding the microbial ecology of recreational waters, especially from non-point source pollution. There is currently little understanding of the impact of source microbiomes such as stormwater or sewage treatment plant outfalls on the overall microbiome of the receiving waters. Current indicator bacteria tests do not identify the potential sources for these bacteria, thus making it impossible to ascertain the source of pathogen indicator bacteria causing exceedance of water quality objectives.

A new technology is available that greatly improves microbial source identification. PhyloChip DNA microarray contains 1.1 million probes that capture representatives of all known, nearly complete 16S rRNA genes in public databases. The PhyloChip can quantify over 59,000 bacterial taxa in a single sample by targeting variations in the 16S rRNA gene. The 16S rRNA gene is universally present in all microbes and small sequence variations within the gene can be used as a “barcode” for bacteria and archaea identification. The analysis quantifies changes in relative abundance of each gene sequence and corresponding bacterial taxa among samples in the study. Recent studies demonstrate the usefulness and performance of this technology for microbial source tracking (Dubinsky et al. 2012, Cao et al. 2013).

To support the development of the Russian River Pathogen TMDL, LBNL used PhyloChip to analyze filters of water quality samples that were collected by the North Coast Regional Quality Control Board. This project focused on microbiological source identification in the middle and lower Russian River watershed.

Monitoring tasks were identified for the following five management questions:

1. What is the spatial variability of the bacterial community?
2. What is the temporal variability of the bacterial community?
3. Do land uses influence the variability of the bacterial community?
4. Does recreational beach use influence the variability of the bacterial community?
5. Do areas with onsite wastewater treatment influence the variability of the bacterial community?

The project consisted of four monitoring tasks designed to answer these questions and determine sources of fecal indicator bacteria. Task 1 assessed spatial and seasonal variability of the microbial community in the Russian River and impaired tributaries. Tasks 2 evaluated the influence of land use on the microbial community. Tasks 3 evaluated the influence of recreational beach use on the microbial community. Task 4 assessed the influence of locations with onsite wastewater treatment systems on the microbial community and diagnostic fecal bacteria.

Methods

Sampling

Two Quality Assurance Project Plans (QAPP) guided the monitoring study. The *Russian River Pathogen Indicator Bacteria TMDL Quality Assurance Project Plan* (Fadness and Butkus 2011) detailed the methods applied for water sample collection and analysis of fecal indicator bacteria *E. coli, Enterococcus,* and total coliform concentrations. The North Coast Regional Water Board Microbiology Laboratory conducted these analyses. The *Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan - Quality Assurance Project Plan* (Butkus 2011) detailed the methods applied for collection and analysis of additional water quality samples. The additional water samples were collected in conjunction with the fecal indicator bacteria TMDL samples. The additional water samples were analyzed for *Bacteroidales* bacteria and stable isotope analyses of nitrate for relative source differences in oxygen ($\delta 18O$) and nitrogen ($\delta 15N$). Frozen samples of water filters used to capture microbial cells were provided to LBNL for PhyloChip analysis. Samples were archived at -80 °C until analysis.

DNA extraction and amplification

DNA was extracted from water filters using the DNA-EZ extraction kit (Generite, New Brunswick, NJ). The 16S rRNA gene was amplified from each DNA extract using PCR with bacterial primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-

GGTTACCTTGTTACGACTT-3') for bacteria. Each PCR reaction contained 1× Ex Taq buffer (Takara Bio Inc., Japan), 0.025 units/µl Ex Taq polymerase, 0.8 mM dNTP mixture, 1.0 µg/µl BSA, and 200 pM each primer and 1 ng genomic DNA (gDNA) as template for fecal samples and 10 ng gDNA for water samples. Each sample was amplified in 8 replicate 25 µl reactions spanning a range of annealing temperatures. PCR conditions were 95°C (3 min), followed by 30 cycles 95°C (30 s), 48-58°C (25 s), 72°C (2 min), followed by a final extension 72°C (10 min). Amplicons from each reaction were pooled for each sample, purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and eluted in 50 µL elution buffer.

PhyloChip analysis

A detailed description of PhyloChip design and validation is available in Hazen et al. (2010 supplementary) and laboratory procedures for PhyloChip analysis are described in Dubinsky et al. (2012). Briefly, replicate PCR was performed to amplify genes encoding 16S rRNA from Bacteria; pooled PCR products were purified then fragmented with DNAaseI; the fragmented products were then labeled with biotin followed by hybridization overnight onto the microarray; the microarray was then stained and scanned to provide raw PhyloChip data in the form of fluorescent image files. Probe intensities were background-subtracted and scaled to quantitative standards (non-16S spike-ins) and outliers were identified as described in Hazen et al. (2010).

Two approaches were used to analyze the fluorescent image files following array scanning. The first approach used the standard operational taxonomic unit (OTU) approach described in Dubinsky et al. (2012). In this approach the presence of 59,316 different bacterial OTUs was determined by positive hybridization of multiple probes that correspond to distinguishing 16S rRNA gene polymorphisms (average of 37 probes/OTU). The hybridization score (HybScore) for an OTU was calculated as the mean intensity of the perfectly matching probes exclusive of the maximum and minimum. Procedures for OTU presence/absence scoring are described in Hazen et al. (2010). This approach yields an inventory of detected OTUs that compose the microbial community.

The second analysis approach considered probe quartet data and is an advancement of the high performing probe-based analysis described in Cao et al. (2013). The probe-based approach uses each of the PhyloChip's 1,015,124 probe features to determine diagnostic sequences for specific fecal sources and detect these targets in environmental samples. This approach was found to be more sensitive and accurate than the OTU approach for fecal source identification in the Source Identification Protocol Project (Cao et al. 2013). In this study we advance this method by analyzing quartets of probes that target the sense, anti-sense, and corresponding mismatch probes of each targeted sequence (Probst et al. 2014). This is the most robust way of determining the presence and abundance of a targeted 16S rRNA gene sequences because it controls for non-specific hybridization and relies on detection of both complimentary DNA strands to increase the performance of the assay.

For this project we re-analyzed data from 80 different fecal sources previously collected by LBNL including all those used in Dubinsky et al (2012) and Cao et al. (2013) for improved sensitivity and specificity. We developed specific quartet-probe profiles for human waste, grazing mammal and shorebird fecal sources. Each reference fecal sample was a composite of individual feces or human waste from a unique location and included sewage, septage, human stool and droppings from cows, horses, deer, elk (grazing animals) and gulls and pelicans (shorebirds). These reference samples were used to define subsets of 16S rRNA gene sequences that are common among samples of a given source type and rare in other fecal sources. These subsets define the diagnostic source identification probes used in this study to probe for fecal signals from human wastes, grazing mammals or shorebirds. Dubinsky et al. (2012) and Cao et al. (2013) found that 20% or greater occurrence of source ID probes for a source was a suitable threshold to detect a source signal in mixtures of sources and dilutions in the complex microbial background of receiving waters.

Statistics

Differences in taxonomic richness among wet and dry period samples in Tasks 1 and 2 were tested using the Mann Whitney U test. Differences among land use types in Task 2 and parcel categories in Task 4 were tested using the Kruskal-Wallis test. Comparisons of overall bacterial community structure were conducted with multivariate statistics using the Bray-Curtis distance metric. Nonmetric Multidimensional Scaling (NMDS) was used in Primer 6 to visualize community differences. Analysis of Similarity (ANOSIM) was used to test whether community structure was different between groups. ANOSIM R values range from 0-1, with values close to 1 indicating strong separation between groups and values close to 0 indicating no significant separation. Similarity Percentage (SIMPER) analysis was used to identify the taxa that were primarily responsible for observed differences in community structure between groups.

III. Task 1: Site Variability

Description

Task 1 was designed to answer the following management questions:

1. What is the spatial variability of the bacterial community in the middle and lower Russian River?
2. What is temporal variability of the bacterial community between wet and dry periods?

Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan were collected on a weekly basis at sixteen (16) different locations along the Russian River and from listed tributaries in the watershed. LBNL conducted PhyloChip analysis on dry period samples collected on August 16-18, 2011 (Table 2-1). Wet period samples were collected for PhyloChip analysis at the same locations on October 5-6, 2011.Wet periods were defined by federal regulation (40 CFR 122.21(g)(7)(ii)) and the USEPA Storm Water Sampling Guidance Document (USEPA 1992) as greater than 0.1 inch and at least 72 hours from the previously measurable (greater than 0.1 inch rainfall) storm event.

Table 2-1. Sample descriptions for Task 1.

Station Name	Sample Code	Latitude	Longitude	Dry sample date	Wet sample date
Alexander Valley Campground	AVC	38.658672	-121.170433	8/16/11	10/6/11
Camp Rose	CR	38.613511	-121.167928	8/16/11	10/6/11
Memorial Beach	MB	38.60465	-121.122922	8/16/11	10/6/11
Steelhead Beach	SB	38.500311	-121.100561	8/16/11	10/6/11
Forestville Access Beach	FAB	38.510331	-121.078803	8/18/11	10/6/11
Johnson's Beach	JB	38.499389	-121.001972	8/18/11	10/6/11
Monte Rio Beach	MRB	38.466258	-122.990628	8/18/11	10/6/11
Commisky Station	CSR	38.882508	-122.944231	8/18/11	10/6/11
Cloverdale River Park	CRP	38.823144	-123.009458	8/18/11	10/6/11
Geyserville @ Highway 28 Bridge	GHB	38.712922	-121.104519	8/18/11	10/6/11
Dutch Bill Creek	DBC	38.463314	-122.990083	8/16/11	10/6/11
Jenner Boat Ramp	JBR	38.449431	-123.115608	8/18/11	10/6/11
Santa Rosa Creek @ Los Alamos Rd.	SRCL	38.458314	-121.36845	8/18/11	10/5/11
Santa Rosa Creek @ Railroad St.	SRCR	38.434813	-122.719683	8/18/11	10/5/11
Laguna de Santa Rosa	LSR	38.407926	-122.818068	8/18/11	10/5/11
Green Valley Creek	GVC	38.480444	-121.091008	8/18/11	10/5/11

Results: Task 1

Spatial and temporal variability of bacterial communities

The taxonomic composition of all wet and dry period samples is summarized in Tables 2-2 and 2-3, respectively, and Figure 2-1. The number of different bacterial taxa, referred to as Operational Taxonomic Units (OTUs), in the Russian River ranged 311 to 583 in the dry period and 310 to 2379 in the wet period. The number of OTUs in impaired tributaries ranged from 531 to 1749 in the dry period and 793 to 1583 in the wet period.

Bacteria communities in the dry period were similar among Russian River beaches from Commisky Station Road to Monte Rio Beach (Figure 2-1). Bacterial communities were mostly composed of Betaproteobacteria (Aquabacterium and Burkholderia), Actinobacteria (Corynebacteriaceae) and non-fecal Bacteroidetes (Flavobacteria) (Table 2-2). All of these taxa are common in freshwater and soil, and include many organisms known for their role in organic matter degradation. The ubiquity of these taxa indicates they are native to the river. Gammaproteobacteria related to Aeromonas were detected with increased frequency at Alexander Valley Campground and downstream sites in the dry period. Aeromonas are known to be ubiquitous in freshwater habitats. It is unclear why they vary among sites during the dry period. The bacterial community at Jenner was the most distinct of all the sites during the dry period (Figure 2-2) and contained >200 Alphaproteobacteria (Pelagibacteria and Rhodobacteraceae) that were not observed at upstream locations (Table 2-2). These Alphaproteobacteria are dominant in coastal oceans and likely occur at Jenner due to the tidal influence of the Pacific Ocean.

In the wet period samples, the bacterial community at beaches between Commisky Station Road to Memorial Beach was similar in composition and structure to dry period samples from the same locations (Table 2-3, Figure 2-2). The community began to diverge at Steelhead Beach and was increasingly distinct moving downstream to Forestville Access, Johnson's and Monte Rio Beaches (Figure 2-2). Divergence at these sites during the wet period was primarily caused by the occurrence of Clostridia that were not found upstream (Table 2-3) or in dry period samples (Table 2-2). At Johnson's Beach and Monte Rio Beach in the wet period, Clostridia, Bacteroidaceae and Verrucomicrobia (Akkermansia species) that are common in human fecal sources were dominant taxa in the microbial community. In addition, large numbers of potentially pathogenic Staphylococcus were found at Johnson's Beach and Monte Rio Beach along with human fecal bacteria. It is important to note that none of the fecal indicator tests used for monitoring (*Enterococcus*, *E. coli*, total coliforms) exceeded water quality limits (CDHS 2011) at Johnson's Beach and Monte Rio Beach (Table 2-3) where numerous fecal-associated Clostridia, Bacteroidaceae, Verrucomicrobia and Staphylococcus were detected.

The wet period sample at Jenner did not contain the dominant Clostridia, Bacteroidales or Staphylococcus found upstream at Monte Rio Beach (Table 2-3), and was more similar in overall community structure to locations upstream of Johnson's Beach (Figure 2-2). The wet period Jenner sample also lacked the marine Alphaproteobacteria that were observed during the dry period (Figure 2-1) indicating little or no marine influence on the microbial community at this time.

Dry period samples from tributaries in impaired watersheds contained greater taxonomic richness than the Russian River, and bacterial community structure in tributaries was generally different than the Russian River (Figure 2-3), mainly due to larger numbers of Alpha-, Beta- and Gammaproteobacteria (Table 2-2, Figure 2-1). These Proteobacteria families are common in soil and freshwater habitats and may be native to these tributaries. Tributary samples that were most distinct from Russian River samples mostly had high counts of fecal indicator bacteria (Figure 2-4). In wet period tributary samples with high fecal indicator counts there were higher numbers of taxa related to Pseudomonas, Enterobacter and Betaproteobacteria but not fecal Bacteroides or Clostridia (Table 2-3). Dry period samples at Green Valley Creek and Santa Rosa Creek exceeded concentration limits for *Enterococcus*, but not *E. coli*, and also contained increased numbers of Proteobacteria taxa (Table 2-2). High numbers of Enterobacteria and Pseudomonas co-occurred in Dutch Bill Creek and Santa Rosa Creek at Los Alamos during wet and dry periods, along with increased detection of Clostridia relative to other dry period samples. It is unclear whether these bacteria are naturally occurring or input from a wastewater or fecal source.

There were no consistent differences in overall community composition samples between all wet and all dry period samples. The difference in taxonomic richness between wet and dry periods was not significant ($p>0.05$). ANOSIM results showed no significant different between the community structure of wet and dry periods samples (ANOSIM $r=0.19$).

Fecal source detection

PhyloChip source detection analysis did not find human, grazing animal or shorebird fecal signal in any dry period samples in the Russian River or impaired tributaries (Figure 2-5).

In the wet period there was human fecal signal at Johnson's Beach and Monte Rio Beach (Figure 2-5). Water samples contained 72-75% of diagnostic human Clostridia, 39-43% of diagnostic Bacteroidales sequences and 54-59% of all 654 16S rRNA gene sequences that are diagnostic for human wastes. These samples also contained high numbers of *Staphylococcus* (Table 2-3, Appendix A). We were not able to refine the source of human fecal signal based on our reference database of sewage, septage and human stool samples because diagnostic bacteria and sewage and septage are largely shared with human stool samples. At Jenner, diagnostic human ID sequences were detected with greater frequency than sites upstream of Johnson's Beach (29% diagnostic human Clostridia), possibly due to the upstream inputs that affected Monte Rio Beach and Johnson's Beach (Figure 2-5). No human fecal sources were indicated at sites upstream of Monte Rio, however taxa related to pathogenic *Yersinia* were detected at Commisky Station, Cloverdale River Park and Geyserville Bridge in both wet and dry periods (Appendix A).

The HuBac qPCR test found a high numbers of human Bacteroidales at Monte Rio Beach in the wet period (Figure 2-6). There was not a strong correlation among PhyloChip human ID results and HuBac results (Figure 2-7). Curiously, there were no exceedances of fecal indicator bacteria at Monte Rio Beach despite the strong indication of human fecal signal by both PhyloChip and HuBac qPCR. These methods detect the presence of DNA, regardless of the viability of the detected organisms. The IDEXX fecal indicator tests measure viable bacteria, and it is possible that wet period samples at Monte Rio and Johnson's Beaches contain non-viable fecal indicator bacteria but high concentrations of human fecal bacteria DNA. For example, there is a positive correlation between IDEXX *E. coli* fecal indicator counts and the relative abundance of *Escherichia* OTUs measured by PhyloChip ($r=0.64$, Figure 2-8), demonstrating a general correspondence between the culture-based FIB assay and PhyloChip DNA quantification. There are, however, several samples in which IDEXX *E. coli* are at or below the detection limit but PhyloChip relative abundances of *Escherichia* OTUs are high (Figure 2-8), indicating that a higher proportion of detected DNA is from non-viable organisms in these particular samples.

During the wet period at Steelhead Beach and Forestville Access Beach, several fecal-associated Lachnospiraceae and Ruminococcaceae were detected indicating influence from a mammalian fecal source. The signal from diagnostic human Bacteroides and Clostridia was not strong enough to indicate a human source (2% and 4%, respectively, Figure 2-5). A possible grazer source was detected at these sites (12% and 19% diagnostic grazer-specific sequences, 23% and 36% of grazer Bacteroides, 7% and 20% of grazer Clostridia). BovBac qPCR also detected a bovine Bacteroides signal at Steelhead Beach and Forestville Access Beach (Figures 2-9 and 2-10). Downstream at Johnson's Beach and Monte Rio Beach a grazer signal was also detected by PhyloChip (17% and 20% diagnostic grazer-specific sequences, 16% and 18% of grazer Bacteroides, 40% and 48% of grazer Clostridia, respectively). We attempted to refine the grazer signal at these sites based on our reference database of cow, horse and deer fecal samples.

Results were inconclusive because fecal signals were weak but there was some indication that a cow source may be affecting these sites (8-13% cow-specific sequences, 2-17% cow Bacteroides, 15-22% cow Clostridia). A possible deer source was also indicated at Johnson's Beach and Monte Rio Beach (7% deer specific sequences, 14-18% deer Bacteroides, 18-22% deer Clostridia). No horse signal was indicated at any of the sites (<3% horse-specific sequences, 0% horse Bacteroides, 0% horse Clostridia).

Dry period samples at Green Valley Creek and Santa Rosa Creek exceeded concentration limits for *Enterococcus*, but not *E. coli*, but few diagnostic fecal bacteria were detected, indicating that human, grazer or shorebird sources were not likely causing exceedances of Enterococcus. In the wet period, Green Valley Creek, Santa Rosa Creek and Laguna de Santa Rosa exceeded all fecal indicator tests but few diagnostic fecal bacteria were detected (Figure 2-5). No grazer sources were indicated for Laguna de Santa Rosa.

Tributary samples contained taxa related to potential pathogens, mostly coliforms including *Klebsiella pneumoniae, Serratia marcescens, Shigella flexneri* (Appendix A). Detection of pathogen related 16S rRNA genes do not necessarily indicate that pathogenic strains are present, but rather that closely related taxa are present that may or may not include the virulent strain. Molecular assays that specifically target pathogenic strains are necessary to confirm their occurrence.

IV. Task 2: Land Use Variability

Description

Task 2 was designed to assess variability among different types of land uses. This task was conducted to assess the relative magnitude and variability of indicator bacteria in waters draining from each of the major land uses found in the Russian River watershed. Definition of land use categories and site selection is described in the Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan Quality Assurance Project Plan (Butkus 2011). Based on the land cover spatial data acreage within the study area five land cover categories were chosen for this assessment:

1. Forest Land
2. Rangeland
3. Agriculture
4. Urban & Residential Sewered areas
5. Residential Non-sewered areas.

In the Russian River Pathogens Pilot Study it was determined that runoff from different land uses exhibited different bacteria levels. The objective of this task was to assess the relative magnitude and variability of bacteria in waters draining from each of the major land uses in the middle and lower Russian River watershed. Task 2 is designed to answer the following management questions:

1. What is the variability of the bacterial community among different land covers?
2. What is the temporal variability of the bacterial community between wet and dry periods?
3. Does land use influence the variability of the bacterial community?

To assess land use variability, sampling was conducted during both wet and dry periods. Samples for the Russian River Pathogen Indicator TMDL Monitoring Plan were collected from October 2011 through June 2012. One of these sampling events was chosen as the dry period sample set and one was chosen as a wet period sample set according to criteria described in the Russian River Pathogen Indicator TMDL Monitoring Plan.

Table 3-1. Sample descriptions for Task 2.

Station ID	Station Name	Land Use Category	Dry sample date	Wet sample date
114UW0048	Abramson Creek	Agriculture	12/9/11	1/21/12
114BL1999	Blucher Creek	Shrubland/Herbaceous	12/9/11	1/21/12
114CO0655	Copeland Creek	Developed Sewered	12/9/11	1/21/12
114CR3673	Crane Creek	Shrubland/Herbaceous	3/5/12	1/21/12
114FO3662	Foss Creek	Developed Sewered	12/9/11	1/20/12
114GO0351	Gossage Creek	Shrubland/Herbaceous	12/9/11	1/21/12
114US1675	Irwin Creek	Developed Onsite Septic	3/5/12	1/21/12
114UD0000	Lambert Creek	Agriculture	3/5/12	1/21/12
114UL3960	Limerick Creek	Developed Onsite Septic	3/5/12	1/21/12
114UM0355	Mays Creek	Forest Land	3/5/12	1/23/12
114PA3647	Palmer Creek	Forest Land	12/9/11	1/20/12
114PI0729	Piner Creek	Developed Sewered	12/9/11	1/21/12
114UT3915	Turner Creek	Developed Onsite Septic	12/9/11	1/21/12
114VB0410	van Buren Creek	Forest Land	12/9/11	1/21/12
114UR3927	Woolsey Creek	Agriculture	3/5/12	1/21/12

Results: Task 2

Variation of bacterial communities among land use types

No significant differences were found among land use types in dry or wet periods for total OTU richness or richness in any taxonomic families (Table 3-2). Median richness in agriculture samples trended higher than other land use types for Comamondaceae, Pseudomonadaceae and total bacterial richness (Table 3-2, Figure 3-1). Median bacterial richness in forest samples trended lower than other land uses during wet periods.

Taxonomic richness was significantly greater during wet periods than dry periods for all land use types (Mann Whitney U test, $p<0.001$) (Figure 3-1). Community structure was significantly different between wet and dry periods for all land use types (ANOSIM Global R = 0.76, p=0.001). Ordination of bacterial abundance data showed that all wet period samples clustered together, regardless of land use type (Figure 3-2). This result indicates that bacterial communities were more sensitive to seasonal effects than land use effects. Differences between dry and wet period samples were primarily due to increased numbers of Bacteroidetes and Proteobacteria (Table 3-4). Bacteroidetes that increased in the wet period consisted of Rikenellaceae, Flavobacteria and Sphingobacteria. These are non-fecal Bacteroidetes taxa that naturally occur in soil and water environments. Most of the Proteobacteria that increased in the wet period were Klebsiella and Pantoea (coliforms) and Pseudomonas. These Proteobacteria are ubiquitous and many different habitats including soils, plant roots, freshwater, sewage and animal guts. Rain may enhance runoff and transport of these bacteria to creeks. The increased detection of these bacteria in all land use types during the wet period indicates that a particular human or animal fecal source is unlikely responsible for their occurrence, and instead that these taxa originate from environmental sources, such as soil or streambanks, that are widespread across all land use types.

The strong separation between dry and wet period samples was correlated with concentrations of fecal indicator bacteria (Figure 3-3). Nearly all exceedances in fecal indicator bacteria occurred in the wet period. The dry period sample from Abramson Creek, an agriculture site, was a notable exception to this pattern. This site had high concentrations of total coliform, but not Enterococcus and *E. coli*, and was distinct in microbial community structure from all other wet and dry period samples (Figure 3-3). There was no apparent affiliation of potential human pathogens to particular land use types, however there was seasonal variation of potential pathogens; taxa related to *Proteus mirabili* were detected in only dry period samples and taxa related to *Shigella flexneri*, *Salmonella enterica*, *Streptococcus* sp. and several *Staphylococcus* were detected in only wet period samples (Appendix A).

Fecal source detection

Dry period samples in all land uses lacked human fecal signal with the exception of Limerick Creek, a developed onsite septic location that had 52% of diagnostic human sequences present including 62% of diagnostic human Bacteroidales (Figure 3-4). This result is consistent with HuBac qPCR results that also found increased human fecal marker in this sample (Figure 3-5). In the Abramson Creek agriculture sample noted for its unique bacterial community (Figure 3-3) the PhyloChip test found evidence of a human source (28% and 21% of human Bacteroides and Clostridia, respectively), consistent with the high human Bacteroides concentration measured in this sample with HuBac qPCR (Figure 3-5).

Diagnostic human fecal sequences were more frequently detected in wet period samples than dry period samples in all land use types but the rate of detection was low (<20%) for most samples, indicating the signal was too weak to conclusively detect a human source. Some wet period samples from developed sewered sites at Copeland and Piner Creeks contained stronger evidence of human fecal signal. At these sites, 25-46% of diagnostic human Bacteroidales were found and 17-25% of human Clostridia. The shrubland sample from Crane Creek also contained a possible human signal (34 and 21% of diagnostic human Bacteroides and Clostridia, respectively). The number of diagnostic human bacteria detected by PhyloChip was weakly correlated with HuBac test results (Figure 3-6).

Dry period samples contained little evidence for grazing animal fecal bacteria (Figure 3-4). In the wet period, evidence for grazer signal was found at Abramson Creek (agriculture) with detection of 41% and 53% of grazer Bacteroides and Clostridia, respectively. All shrubland sites contained evidence of grazer fecal signal (34-41% and 31-41% of grazer Bacteroides and Clostridia, respectively). In addition, there was indication of possible grazer signal at Turner Creek (developed onsite septic) and Copeland Creek (developed sewered) (32% and 41% of grazer Bacteroides, and 37% and 41% of grazer Clostridia, respectively). These results are consistent with BovBac Bacteroides concentrations (Figures 3-7 and 3-8). It should be noted that these targets have not been thoroughly tested for cross-reactivity in non-fecal samples such as soils, sediments and decaying vegetation, so these results should be treated with caution. Further refinement of the grazer signal with cow, horse and deer specific probes did not yield conclusive results.

V. Task 3: Recreational Use Variability

Description

The task was designed to assess the relative magnitude and variability of indicator bacteria levels that may be associated with increased human recreation use on weekends. Water samples were collected and analyzed to assess the local impact of recreational activities on indicator bacteria levels at public beaches. Waters samples were collected at two beaches on the Russian River that experience large amounts of public use: Johnson's Beach in Guerneville and Monte Rio Beach in Monte Rio. Samples analyzed by PhyloChip analysis were collected for five consecutive days during September 22-26, 2011 to assess daily variability. Sample collection dates bracketed the Russian River Jazz & Blues Festival and the Russian River Cleanup to capture variability in microbial communities due to the elevated recreational use.

Table 4-1. Sample descriptions for Task 4

Station ID	Station Name	Location	Latitude	Longitude
114RR1325	Johnson's Beach	Church Street	38.499389	-121.001972
114RR0898	Monte Rio Beach	Bohemian Hwy	38.466258	-122.990628

Results: Task 3

In the recreational beach use study, there was human fecal signal at Johnson's Beach on the fifth day of monitoring (9/26/2011). This sample was different in composition from other Johnson's Beach and Monte Rio samples (Figure 4-1 and 4-2) and contained high numbers of Clostridia (Table 4-2). This sample contained 468 OTUs of fecal-associated Clostridia in the Lachnospiraceae and Ruminococcus, compared with 0 to 6 in non-exceedance samples. This sample contained 50% of diagnostic human fecal targets, including 77% of human Clostridia and 30% of human Bacteroidales (Figure 4-3). There was a weaker indication of grazing animal feces (18% of detected targets) and no indication of shorebird signal (Figure 4-3).

The 9/26 Johnson's Beach sample with probable human fecal signal was associated with an *Enterococcus* concentration that marginally exceeded the water quality limit (63 MPN/100mL) (CDHS 2011) but the *E. coli* concentration was below the exceedance limit. In this instance the *E. coli* test may have missed the potential risk. Likewise, the HuBac qPCR test did not indicate an elevated risk in this sample (Figure 4-4). It important to note that human fecal signal and high numbers of *Staphylococcus* were detected at Johnson's Beach as well as Monte Rio Beach in the Site Variability study (Task 1) but were similarly not affiliated with exceeding concentrations of fecal indicator bacteria. Two *Staphylococcus* OTUs were detected when fecal bacteria were present during the recreational study (Appendix A).

VI. Task 4: On-Site Wastewater Treatment Systems Study

Description

Task 4 was designed to answer the following management question:

1. Do catchments with high density of on-site wastewater treatment (OSWT) systems contribute pathogenic indicator bacteria from human sources?

The assessment for the Russian River TMDL monitoring data collected in 2011-2012 (NCRWQCB 2012) identified the need to conduct a more robust assessment of the human contribution to exceedance of pathogenic indicator bacteria criteria. Areas that drain from catchments that have a high density of OSWT systems were compared to catchments with a low density of OSWT systems. Nine (9) sample locations were selected for both high-density and low-density catchments throughout the study area. Wet weather samples were collected only from ephemeral stream locations. Samples analyzed for PhyloChip are listed in Table 5-1.

Sample blanks were collected during each sample event (5 blanks). For each of the blank samples, sterile water was poured into the sample container in the field. For each of the PhyloChip samples, sterile water was poured into the sample container in the field and subsequently filtered in the North Coast Regional Water Board laboratory.

Table 5-1. Sample descriptions for Task 4.

Site	Category	Sample date
Site 01	High Parcel Density - High Septic Risk	03/06/13
Site 01	High Parcel Density - High Septic Risk	03/20/13
Site 02	High Parcel Density - High Septic Risk	03/20/13
Site 02	High Parcel Density - High Septic Risk	04/04/13
Site 02	High Parcel Density - High Septic Risk	12/3/12
Site 03	High Parcel Density - High Septic Risk	03/06/13
Site 03	High Parcel Density - High Septic Risk	03/20/13
Site 03	High Parcel Density - High Septic Risk	04/04/13
Site 03	High Parcel Density - High Septic Risk	12/3/12
Site 04	High Parcel Density - Low Septic Risk	03/06/13
Site 05	High Parcel Density - Low Septic Risk	02/19/13
Site 05	High Parcel Density - Low Septic Risk	03/06/13
Site 05	High Parcel Density - Low Septic Risk	04/04/13
Site 06	High Parcel Density - Low Septic Risk	04/04/13
Site 06	High Parcel Density - Low Septic Risk	12/3/12
Site 07	Low Parcel Density - High Septic Risk	04/04/13
Site 07	Low Parcel Density - High Septic Risk	12/3/12
Site 08	Low Parcel Density - High Septic Risk	04/04/13
Site 08	Low Parcel Density - High Septic Risk	12/3/12
Site 09	Low Parcel Density - High Septic Risk	12/3/12
Site 10	Low Parcel Density - Low Septic Risk	04/04/13
Site 10	Low Parcel Density - Low Septic Risk	12/3/12
Site 11	Low Parcel Density - Low Septic Risk	02/19/13
Site 12	Low Parcel Density - Low Septic Risk	04/04/13
Site 12	Low Parcel Density - Low Septic Risk	12/3/12
Site 13	Catchments of Interest	03/20/13
Site 13	Catchments of Interest	04/04/13
Site 14	Catchments of Interest	12/3/12
Site 15	Catchments of Interest	03/06/13
Site 15	Catchments of Interest	03/20/13

Results: Task 4

There were no significant differences in bacterial communities associated with parcel density or septic risk (Figure 5-1). The concentrations of fecal indicator bacteria in different risk categories or other catchments of interest were not associated with any trends in community structure (Figure 5-2) or composition (Figures 5-3 to 5-5). There were no trends in community composition or structure associated with samples that exceeded concentration limits of *Enterococcus* fecal indicators but had low concentrations of *E. coli* fecal indicators.

Human fecal signal was not detected at sites in areas with both high parcel density and high septic risk (<10% of diagnostic human fecal bacteria) (Figure 5-6). In areas with high parcel density and low septic risk, one site (Site 5) was had a human fecal signal on two sampling dates (Figure 5-6). These samples contained 64-82% of Bacteroidales sequences and 34-44% of Clostridia sequences that are diagnostic of human fecal waste. No human fecal signal was detected at low parcel density sites with both low and high septic risk (Figure 5-7). In the three additional catchments of interest that were analyzed, only site 14 had a strong human fecal signal with 94% and 96% of diagnostic human Bacteroidales and Clostridia, respectively (Figure 5-8).

Comparisons between PhyloChip results and HuBac qPCR results showed PhyloChip detection of human fecal signal was associated with higher numbers of HuBac Bacteroides targets (Figures 5-9 to 5-12). However, HuBac Bacteroides were measured in several samples where PhyloChip detected no human fecal signal. The reason for this discrepancy requires further investigation, but the HuBac assay is known to have very low specificity to human fecal sources (Shanks et al. 2010), consistent with its prolific detection of Bacteroides in most samples of the Russian River watershed analyzed in Tasks 1-4. The lack of HuBac specificity may explain discrepancies with PhyloChip results.

VII. Summary and Conclusions

Task 1: Site Variability

- In the dry period, bacterial communities were similar at all sites along the middle and lower reaches of the Russian River with the exception of Jenner where there was a marine influence on the bacterial community. In the wet period, bacterial communities in samples upstream Steelhead Beach were similar to dry period samples.

- Human fecal signal was found at Johnson's Beach and Monte Rio Beach during the wet period. Water at these beaches contained high numbers of human-associated *Bacteroidales* and *Clostridia*, as well as high numbers of *Staphylococcus*.

- Neither *Enterococcus* nor *E. coli* fecal indicator tests exceeded water quality limits when human fecal bacteria and Staphylococcus were detected at Johnson's Beach and Monte Rio Beach.

- Grazer fecal bacteria were detected during the wet period at Steelhead Beach and Forestville Access Beach. Cattle or deer may be a source for these bacteria.

- No human or grazing animal fecal signal was found at sites upstream of Steelhead Beach. *Yersinia* sp. were detected in both wet and dry periods at Commisky Station, Cloverdale River Park and Geyserville Bridge

- Bacterial communities in impaired tributaries typically contained nearly twice the number of bacterial taxa as the Russian River including high numbers of coliforms (Enterobacteria) and Pseudomonas. No human or animal fecal signal was detected in tributaries with high fecal indicator counts.

Task 2: Land Use Variability

- No significant differences in bacterial communities were found among land use types in dry or wet periods. Median richness in agriculture samples trended higher than other land use types for Comamondaceae, Pseudomonadaceae and total bacterial richness (Table 3-2, Figure 3-1)

- Taxonomic richness in all land uses was significantly greater during wet periods than dry periods for all land use types and associated with high counts of fecal indicator bacteria. Wet period bacterial communities were similar among all land use types and contained large numbers of non-fecal Bacteroidetes, and Proteobacteria that were mainly Enterobacteria (coliforms) and Pseudomonas.

- Human fecal signal was not detected in dry period samples with the exception of Limerick Creek, a developed onsite septic location. Grazing animal signal was not found in any land use samples during the dry period.

- Wet period samples from developed sewered sites contained possible signal from human fecal bacteria. Several wet period samples from different land use categories contained signal from grazing animal fecal bacteria.

- Detection of potential pathogens was not associated with land use but did vary seasonally. Taxa related to *Proteus mirabili* were detected in only dry period samples and taxa related to *Shigella flexneri, Salmonella enterica*, *Streptococcus* sp. and several *Staphylococcus* in only wet period samples.

Task 3: Recreational Use Variability

- Human fecal bacteria were detected at Johnson's Beach on the fifth day of monitoring during the period of heavy recreational use.

- The sample with human fecal signal was associated with an *Enterococcus* concentration that marginally exceeded the water quality limit and an *E. coli* concentration that was below the exceedance limit.

Task 4: Effects of Onsite Wastewater Treatment Systems

- There were no significant differences in bacterial communities associated with parcel density or septic risk

- There were no trends in bacterial communities associated with samples that exceeded concentration limits of *Enterococcus* fecal indicators but had low concentrations *E. coli* fecal indicators.

- No sites in areas with both high parcel density and high septic risk contained evidence of human fecal signal.

- In areas with high parcel density and low septic risk, one site (Site 5) was found to have probable human fecal signal on two sampling dates.

- No human fecal signal was detected at low parcel density sites with both low and high septic risk. In the three additional catchments of interest that were analyzed, site 14 had a strong human fecal signal.

Conclusions

Wet periods have strong effect on the bacterial community at Russian River beaches in the lower watershed and on creeks in all land use types. The PhyloChip assay detected human fecal signal at Johnson's Beach and Monte Rio Beach, and indicated possible risk from pathogenic *Staphylococcus* at these locations during wet periods. Recreational beach use was also associated with human fecal signal. The inconsistency of conventional fecal indicator tests in detecting these risks warrants further investigation.

At other locations upstream in the Russian River, in impaired tributaries, and throughout the surrounding watershed, samples with exceedances in fecal indicator bacteria were frequently unassociated with fecal bacterial taxa. Similarly, many exceedances in areas with high septic risks and high numbers of fecal indicator bacteria had no indication of fecal signal in the microbial community. These results indicate that non-fecal sources are likely supplying *Enterococcus*, *E. coli* and other coliforms to monitored waters.

The absence of significant bacterial community signatures for different land use types indicates that generalizable land use signatures may not be available for source tracking on a landscape scale. There were, however, distinct bacterial communities measured in different creeks that may be useful for tracking downstream influence. In addition, the use of microbial community analysis holds great potential to further identify potential non-fecal sources of fecal indicator bacteria that appear to be important in the Russian River watershed.

VIII. References

Cao, Y., L.C. Van De Werfhorst, E. A. Dubinsky, B. D. Badgley, M. J. Sadowsky, G. L. Andersen, J. F. Griffith and P. A. Holden. 2013. Evaluation of Molecular Community Analysis Methods for Discerning Fecal Sources and Human Waste. *Water Research* 47: 6862-6872.

Butkus, S. 2011. Russian River Pathogen Indicator Bacteria TMDL – Supplemental Sampling Plan - Quality Assurance Project Plan. Dated November 16, 2011. North Coast Regional Water Quality Control Board, Santa Rosa, CA.

California Department of Health Services (CDHS). 2011. Draft Guidance for Fresh Water Beaches. Last Update: January 2011. Initial Draft: November 1997. California Department of Health Services Division of Drinking Water and Environmental Management.

Dubinsky, E.A., L. Esmaili, J. R. Hulls, Y. Cao, J. F. Griffith and G. L. Andersen. 2012. Application of phylogenetic microarray analysis to discriminate sources of fecal pollution. *Environmental Science & Technology* 46:4340-4347.

Fadness, R. and S. Butkus. 2011. Russian River Pathogen Indicator Bacteria TMDL – Quality Assurance Project Plan. Dated May 19, 2011. North Coast Regional Water Quality Control Board, Santa Rosa, CA.

Hazen, T. C., E. A. Dubinsky, T. Z. DeSantis, G. L. Andersen, Y. M. Piceno, et al. 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* 330:204-208.

North Coast Regional Water Quality Control Board (NCRWQCB). 2012. Russian River Pathogen TMDL 2011-2012 Monitoring Report. North Coast Regional Water Quality Control Board. Santa Rosa, CA.

Probst, A.J., P. Y. Lum, B. John, E. A. Dubinsky, Y. M. Piceno, L. M. Tom, G. L. Andersen, Z. He and T. Z. DeSantis. 2014. Microarray of 16S rRNA Gene Probes for Quantifying Population Differences Across Microbiome Samples. Pages 99-119 *in* Microarrays for Microbial Community Analysis (in press).

Shanks, O.C., K. White, C. A. Kelty, M. Sivaganesan, J. Blannon, M. Meckes, M. Varma and R. A. Haugland. 2010. Performance of PCR-Based Assays Targeting Bacteroidales Genetic Markers of Human Fecal Pollution in Sewage and Fecal Samples. *Environmental Science & Technology* 2010 44: 6281–6288.

U.S. Environmental Protection Agency. (USEPA) 1992. NPDES Stormwater Sampling Guidance Document. EPA 833-B-92-001. Washington, D.C.

Table 2-2. Taxonomic richness of bacteria in the Russian River and tributaries during the dry period sampling. Values are the number of detected OTUs in 20 taxonomic families that had highest maximum OTU richness. Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison. Fecal indicator exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

DRY PERIOD	Russian River Beaches											Tributaries				
Taxonomic Family	Commisky Station Rd.	Cloverdale River Park	Geyserville Bridge	Alexander Valley Camp.	Camp Rose	Memorial Beach	Steelhead Beach	Forestville River Access	Johnson's Beach	Monte Rio Beach	Jenner Boat Ramp	Dutch Bill Creek	Green Valley Creek	Laguna de Santa Rosa	Santa Rosa Ck @ Los Alamos	Santa Rosa Ck @ Railroad
Actinobacteria ; Corynebacteriaceae	42	52	54	42	47	71	59	55	48	59	45	79	80	71	33	59
Bacteroidia ; Bacteroidaceae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Flavobacteria ; Flavobacteriaceae	34	23	34	28	23	23	27	18	10	10	43	47	53	20	29	31
Bacilli ; Staphylococcaceae	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1
Clostridia ; Clostridiales Family XI.	0	0	0	0	0	0	0	0	2	0	0	7	1	0	0	0
Clostridia ; Lachnospiraceae	0	0	0	2	0	1	0	0	0	0	0	28	3	0	5	1
Clostridia ; Ruminococcaceae	0	0	0	0	1	1	0	0	2	2	0	27	3	0	10	1
Alphaproteobacteria ; Rhodobacteraceae	0	2	3	4	5	14	9	9	9	8	65	16	11	11	11	6
Alphaproteobacteria ; Rhodospirillaceae	2	1	2	3	8	4	2	2	4	3	5	37	11	4	15	3
Alphaproteobacteria ; Pelagibacteraceae	1	0	0	0	0	0	4	3	3	0	173	4	2	6	2	0
Alphaproteobacteria ; Sphingomonadaceae	1	1	4	3	2	4	3	0	3	7	6	28	27	2	18	4
Betaproteobacteria ; Aquabacteriaceae	70	44	89	55	100	59	97	108	43	60	36	66	197	127	78	124
Betaproteobacteria ; Burkholderiaceae	7	7	5	10	4	16	9	8	13	10	6	49	28	16	21	16
Betaproteobacteria ; Comamonadaceae	60	65	100	62	105	121	82	89	72	101	47	101	139	176	144	94
Betaproteobacteria ; Oxalobacteraceae	0	0	2	5	1	3	2	3	7	11	1	46	19	7	22	3
Gammaproteobacteria ; Aeromonadaceae	2	9	0	46	24	44	83	10	3	17	0	21	15	0	27	29
Gammaproteobacteria ; Enterobacteriaceae	3	13	10	12	3	13	6	3	2	1	19	161	8	3	91	5
Gammaproteobacteria ; Moraxellaceae	0	0	0	0	0	0	0	0	0	0	0	9	1	0	5	0
Gammaproteobacteria ; Pseudomonadaceae	1	0	1	5	1	3	2	0	0	2	5	125	13	5	86	6
Verrucomicrobiae ; Verrucomicrobiaceae	3	2	3	2	5	7	6	3	4	7	5	5	7	3	0	3
ALL BACTERIAL FAMILIES	311	321	413	454	420	550	473	411	337	439	583	1749	946	691	974	531
Enterococcus (MPN/100mL)	52	41	10	31	20	10	36	41	10	10	10	10	187	52	379	97
E. coli (MPN/100mL)	10	10	10	10	20	10	10	10	10	10	10	10	31	74	192	246
Total coliform (MPN/100mL)	1789	3076	1632.5	2625	2018.5	3076	697	878	991	1439	6867	341	1086	1500	2844.5	4611
Ratio *Enterococcus/E.coli* in exceedances	-	-	-	-	-	-	-	-	-	-	-	-	6.0	-	2.0	0.4

Table 2-3. Taxonomic richness of bacteria in the Russian River and tributaries during the wet period sampling. Values are the number of detected OTUs in 20 taxonomic families that had highest maximum OTU richness. Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison. Fecal indicator exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

WET PERIOD	Russian River Beaches											Tributaries				
Taxonomic Family	Commisky Station Rd.	Cloverdale River Park	Geyserville Bridge	Alexander Valley Camp	Camp Rose	Memorial Beach	Steelhead Beach	Forestville River Access	Johnson's Beach	Monte Rio Beach	Jenner Boat Ramp	Dutch Bill Creek	Green Valley Creek	Laguna de Santa Rosa	Santa Rosa Ck Los Alamos	Santa Rosa Ck Railroad
Actinobacteria ; Corynebacteriaceae	49	39	53	41	49	48	45	61	82	117	52	23	74	49	53	39
Bacteroidia ; Bacteroidaceae	0	0	1	0	1	0	0	0	16	33	0	0	0	0	0	0
Flavobacteria ; Flavobacteriaceae	47	28	59	20	31	20	60	82	69	42	42	42	80	115	78	73
Bacilli ; Staphylococcaceae	1	2	1	1	1	1	1	1	115	260	1	0	1	1	1	1
Clostridia ; Clostridiales Family XI.	0	0	0	0	0	0	0	1	49	81	1	2	0	1	3	0
Clostridia ; Lachnospiraceae	1	0	0	1	0	0	39	44	173	546	0	6	2	0	5	1
Clostridia ; Ruminococcaceae	0	0	2	0	0	0	7	11	76	227	0	4	0	0	3	1
Alphaproteobacteria ; Rhodobacteraceae	7	3	8	3	3	4	5	7	18	17	13	12	9	11	19	7
Alphaproteobacteria ; Rhodospirillaceae	3	4	5	0	1	1	3	4	9	10	0	13	6	1	18	6
Alphaproteobacteria ; Pelagibacteraceae	8	4	10	0	1	0	0	2	4	2	3	0	0	0	0	0
Alphaproteobacteria ; Sphingomonadaceae	6	5	12	1	2	2	9	11	17	10	10	30	23	33	42	14
Betaproteobacteria ; Aquabacteriaceae	107	84	182	87	53	86	86	99	172	171	80	69	157	183	177	152
Betaproteobacteria ; Burkholderiaceae	5	7	9	6	9	9	7	15	12	12	8	26	15	26	24	35
Betaproteobacteria ; Comamonadaceae	81	61	119	49	98	82	73	158	245	249	72	75	154	271	282	293
Betaproteobacteria ; Oxalobacteraceae	8	2	14	2	5	4	4	9	25	22	7	29	22	36	31	68
Gammaproteobacteria ; Aeromonadaceae	17	27	71	32	11	22	4	11	51	18	0	14	2	62	59	59
Gammaproteobacteria ; Enterobacteriaceae	24	8	15	2	8	3	5	11	46	57	3	48	5	192	106	256
Gammaproteobacteria ; Moraxellaceae	0	0	2	1	0	0	0	0	2	30	1	3	3	2	4	5
Gammaproteobacteria ; Pseudomonadaceae	9	0	10	0	2	1	3	7	9	8	7	86	9	114	154	246
Verrucomicrobiae ; Verrucomicrobiaceae	3	2	4	4	3	4	4	7	24	66	5	4	2	1	7	2
ALL BACTERIAL FAMILIES	491	381	820	310	389	354	497	754	1475	2379	390	859	793	1305	1583	1566
Enterococcus (MPN/100mL)	959	110	98	20	20	109	26	20	10	10	20	31	987	5794	504	812
E. coli (MPN/100mL)	767	254	148	74	63	447	36	231	63	75	121	10	1918	15531	1455	2014
Total coliform (MPN/100mL)	24196	4009	3076	1259	1100	4229	1248	1223	1071	1467	4478	4352	24196	24196	10462	24196
Ratio *Enterococcus/E.coli* in exceedances	1.3	0.4	0.7	-	-	0.2	-	-	-	-	-	-	0.5	0.4	0.3	0.4

Table 3-2. Taxonomic richness of bacteria in different land use types during dry and wet periods. Values are the median number of detected OTUs in 20 taxonomic families that had highest maximum OTU richness. Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison. Fecal indicator exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

	Dry Period					Wet Period				
Taxonomic Family	**Forest**	**Shrubland**	**Agriculture**	**Septic**	**Sewered**	**Forest**	**Shrubland**	**Agriculture**	**Septic**	**Sewered**
Acidobacteria ; Acidobacteriaceae	10	0	5	4	1	48	28	21	28	31
Actinobacteria ; Corynebacteriaceae	29	56	55	43	28	55	58	53	64	67
Actinobacteria ; Micrococcaceae	0	1	0	0	0	6	10	36	11	2
Bacteroidia ; RikenellaceaeII	6	5	16	4	5	28	60	68	57	12
Flavobacteria ; Flavobacteriaceae	27	57	58	50	29	99	290	194	137	108
Bacilli ; Bacillaceae	1	1	3	2	0	4	22	32	17	11
Bacilli ; Planococcaceae	0	0	0	0	0	0	10	38	9	5
Bacilli ; Streptococcaceae	2	0	0	0	0	1	1	9	5	33
Clostridia ; Lachnospiraceae	4	3	6	7	4	6	54	75	31	9
Clostridia ; Ruminococcaceae	6	2	6	3	2	10	53	57	34	2
Alphaproteobacteria ; Rhodospirillaceae	9	5	10	6	3	40	34	23	36	34
Alphaproteobacteria ; Sphingomonadaceae	16	3	7	5	4	44	54	42	54	52
Betaproteobacteria ; Aquabacteriaceae	69	180	147	130	143	173	203	165	180	177
Betaproteobacteria ; Burkholderiaceae	16	10	10	16	12	50	63	41	51	55
Betaproteobacteria ; Comamonadaceae	80	112	152	85	117	113	284	315	164	139
Betaproteobacteria ; Oxalobacteraceae	23	15	26	36	22	55	63	74	62	66
Gammaproteobacteria ; Enterobacteriaceae	14	8	4	51	4	46	175	299	236	167
Gammaproteobacteria ; Moraxellaceae	3	1	5	2	0	6	5	33	8	9
Gammaproteobacteria ; Pseudomonadaceae	71	22	110	54	8	301	438	354	409	341
Gammaproteobacteria ; Xanthomonadaceae	5	1	4	3	0	29	54	57	62	33
ALL BACTERIAL FAMILIES	654	623	1077	677	643	1715	2466	2796	2442	2096

Table 3-4. Characteristic taxa in wet period samples. Listed OTUs were the top 10% of OTUs that accounted for distinctions between wet and dry period samples determined by SIMPER analysis. Taxa in families with 10 or more total OTUs are shown.

Phylum	Class	Order	Family	Genus	OTU count
Bacteroidetes	Bacteroidia	Bacteroidales	RikenellaceaeII	unclassified	17
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Aequorivita	1
				Capnocytophaga	1
				Chryseobacterium	18
				Flavobacterium	1
				unclassified	1
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	12
				Sphingobacterium	4
				unclassified	6
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	1
				Massilia	3
				unclassified	8
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	1
				Enterobacter	1
				Erwinia	3
				Klebsiella	19
				Leclercia	1
				Pantoea	34
				Raoultella	2
				Serratia	1
				unclassified	33
		Pseudomonadales	Pseudomonadaceae	Pseudomonas	172
		Xanthomonadales	Xanthomonadaceae	Dyella	2
				Luteibacter	1
				Rhodanobacter	5
				Stenotrophomonas	2
				Thermomonas	1
				unclassified	2
				Xanthomonas	2

Table 4-2. Taxonomic richness of bacteria in at Johnson's Beach and Monte Rio Beach during a period of heavy recreational use. Values are the median number of detected OTUs in 20 taxonomic families that had highest maximum OTU richness. Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison. Fecal indicator exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

	Johnson's Beach					Monte Rio Beach				
Taxonomic Family	**9/22**	**9/23**	**9/24**	**9/25**	**9/26**	**9/22**	**9/23**	**9/24**	**9/25**	**9/26**
Actinobacteria ; ACK-M1	12	18	16	14	14	14	14	21	19	19
Actinobacteria ; Corynebacteriaceae	60	52	65	77	81	62	48	58	89	55
Actinobacteria ; Microbacteriaceae	10	7	11	11	13	10	8	8	12	6
Flavobacteria ; Flavobacteriaceae	9	12	18	14	11	15	10	15	21	14
Sphingobacteria ; Chitinophagaceae	4	9	16	9	6	4	6	10	12	3
Clostridia ; Clostridiales	0	0	6	2	15	0	0	1	5	0
Clostridia ; Lachnospiraceae	0	0	0	1	218	0	0	2	4	2
Clostridia ; Ruminococcaceae	1	0	2	2	268	2	0	0	2	0
Alphaproteobacteria ; Rhodobacteraceae	11	11	12	12	13	7	5	8	15	5
Betaproteobacteria ; Aquabacteriaceae	52	99	158	127	58	152	93	135	196	106
Betaproteobacteria ; Burkholderiaceae	14	9	10	15	11	4	6	12	19	11
Betaproteobacteria ; Comamonadaceae	69	76	156	85	79	87	65	98	136	91
Betaproteobacteria ; Oxalobacteraceae	12	2	7	11	9	10	5	9	17	10
Betaproteobacteria ; Methylophilaceae	11	7	12	10	7	5	6	10	12	6
Betaproteobacteria ; Rhodocyclaceae	6	7	10	7	11	11	10	11	18	6
Gammaproteobacteria ; Aeromonadaceae	6	0	7	10	5	19	3	11	42	3
Verrucomicrobiae ; Verrucomicrobiaceae	4	5	7	7	9	4	3	6	11	4
ALL BACTERIAL FAMILIES	424	412	683	568	997	543	388	548	862	430
Enterococcus (MPN/100mL)	58	30	37	30	63	11	9	18	18	21
E. coli (MPN/100mL)	20	16	24	7	22	3	22	22	15	22
Total coliform (MPN/100mL)	921	1046	980	816	1553	1733	1300	1986	1300	1300

Table 5-2. Taxonomic richness of bacteria in high parcel density samples with high and low septic risk. Values are the number of detected OTUs summarized by taxonomic family. The most taxonomically rich families are shown (>30 OTUs in at least one sample). Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison and exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

	High Parcel Density - High Septic Risk									High Parcel Density - Low Septic Risk					
	Site 01	Site 01	Site 02	Site 02	Site 02	Site 03	Site 03	Site 03	Site 03	Site 04	Site 05	Site 05	Site 05	Site 06	Site 06
Taxonomic Family	**3/6/13**	**3/20/13**	**12/3/12**	**3/20/13**	**4/4/13**	**12/3/12**	**3/6/13**	**3/20/13**	**4/4/13**	**3/6/13**	**2/19/13**	**3/6/13**	**4/4/13**	**12/3/12**	**4/4/13**
Acidobacteria ; Acidobacteriaceae	38	26	31	15	11	31	6	11	13	18	9	13	13	22	14
Actinobacteria ; Corynebacteriaceae	71	61	78	51	57	43	31	54	53	53	45	55	49	50	65
Actinobacteria ; Micrococcaceae	5	0	0	2	0	0	0	9	0	0	0	2	0	0	30
Bacteroidia ; Bacteroidaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacteroidia ; Prevotellaceae	0	0	0	0	0	0	0	1	4	13	0	9	17	1	0
Bacteroidia ; RikenellaceaeII	19	12	6	8	6	6	5	6	14	9	3	19	34	6	20
Flavobacteria ; Flavobacteriaceae	153	79	58	97	96	42	32	67	71	58	66	78	105	44	151
Sphingobacteria ; Chitinophagaceae	37	31	20	26	25	12	14	17	12	24	8	6	11	10	29
Nostocophycideae ; Nostocaceae	1	0	1	1	1	0	0	0	2	1	0	0	1	0	0
Bacilli ; Bacillaceae	9	6	6	4	1	4	1	2	3	2	2	8	13	6	8
Clostridia ; Lachnospiraceae	14	7	4	1	4	3	4	4	8	5	5	15	15	5	4
Clostridia ; Ruminococcaceae	9	4	3	2	1	3	1	6	5	4	0	6	15	6	3
Planctomycea ; Planctomycetaceae	40	33	15	7	5	5	9	11	4	3	9	9	9	12	13
Alphaproteobacteria ; Bradyrhizobiaceae	46	16	28	4	2	9	0	3	1	0	1	2	3	8	5
Alphaproteobacteria ; Rhizobiaceae	33	4	39	9	25	0	1	3	1	0	1	0	1	0	1
Alphaproteobacteria ; Rhodospirillaceae	47	25	42	26	24	27	16	25	26	24	8	11	17	19	12
Alphaproteobacteria ; Sphingomonadaceae	61	45	88	25	19	9	1	20	14	8	1	6	10	2	34
Betaproteobacteria ; Aquabacteriaceae	240	230	196	186	202	162	126	149	180	195	169	172	173	146	200
Betaproteobacteria ; Burkholderiaceae	42	20	44	29	34	27	11	38	48	17	23	23	29	8	22
Betaproteobacteria ; Comamonadaceae	171	155	83	86	133	52	46	133	129	77	87	98	131	39	162
Betaproteobacteria ; Oxalobacteraceae	34	17	45	73	71	35	28	67	64	43	40	53	64	30	61
Betaproteobacteria ; Rhodocyclaceae	29	24	9	10	11	7	9	31	25	11	10	12	23	5	25
Gammaproteobacteria ; Aeromonadaceae	34	45	1	14	1	24	7	31	22	10	12	32	73	14	33
Gammaproteobacteria ; Enterobacteriaceae	76	41	97	158	150	34	37	128	73	32	92	75	130	17	250
Gammaproteobacteria ; Pseudomonadaceae	222	153	130	415	441	70	166	432	342	153	302	266	373	60	482
Gammaproteobacteria ; Xanthomonadaceae	28	18	11	44	43	4	6	47	19	4	5	4	17	3	53
Verrucomicrobiae ; Verrucomicrobiaceae	12	11	6	4	1	1	5	1	2	5	3	1	1	4	2
ALL BACTERIAL FAMILIES	2188	1538	1476	1603	1670	991	809	1648	1501	1045	1066	1252	1671	845	2030
Enterococcus (MPN/100mL)	220	20	384	>24,196	5172	295	432	216	613	12997	86	3873	4950	211	41060
E. coli (MPN/100mL)	3179	51	1019	152	187	158	160	3654	146	2613	393	1664	4892	246	2755
Total coliform (MPN/100mL)	6588	1337	>24,196	>24,196	>24,196	4106	9804	>24,196	12997	>24,196	7933	>24,196	98040	6488	>24,196
Ratio *Enterococcus/E.coli*	0.1	0.4	0.4	159.2	27.7	1.9	2.7	0.1	4.2	5.0	0.2	2.3	1.0	0.9	14.9

Table 5-3. Taxonomic richness of bacteria in low parcel density samples with high and low septic risk, and additional catchments of interest. Values are the number of detected OTUs summarized by taxonomic family. The most taxonomically rich families are shown (>30 OTUs in at least one sample). Family data are highlighted as follows: no shading (<10 OTUs), green (10-50 OTUs), yellow (51-150 OTUs), red (>150 OTUs). Results of standard fecal indicator tests are shown for comparison and exceedances are shaded in gray (*Enterococcus* > 61 MPN/100mL, *E. coli* > 235 MPN/100mL, total coliforms >10,000 MPN/100mL).

	Low Parcel Density - High Septic Risk					Low Parcel Density - Low Septic Risk					Catchments of Interest				
Taxonomic Family	**Site 07 12/3/12**	**Site 07 4/4/13**	**Site 08 12/3/12**	**Site 08 4/4/13**	**Site 09 12/3/12**	**Site 10 12/3/12**	**Site 10 4/4/13**	**Site 11 2/19/13**	**Site 12 12/3/12**	**Site 12 4/4/13**	**Site 13 3/20/13**	**Site 13 4/4/13**	**Site 14 12/3/12**	**Site 15 3/6/13**	**Site 15 3/20/13**
Acidobacteria ; Acidobacteriaceae	16	41	7	19	63	9	9	4	23	17	30	40	15	27	52
Actinobacteria ; Corynebacteriaceae	44	62	31	75	74	44	58	52	57	62	62	71	58	54	86
Actinobacteria ; Micrococcaceae	0	0	0	43	30	0	0	0	2	1	5	8	0	0	27
Bacteroidia ; Bacteroidaceae	0	0	0	1	0	0	0	0	0	0	1	0	34	0	0
Bacteroidia ; Prevotellaceae	0	0	0	2	0	0	0	0	0	0	2	0	208	11	0
Bacteroidia ; RikenellaceaeII	2	18	2	17	13	7	38	0	5	14	20	29	25	3	15
Flavobacteria ; Flavobacteriaceae	19	45	18	283	112	44	138	63	35	154	85	166	76	17	133
Sphingobacteria ; Chitinophagaceae	4	22	4	39	38	3	11	11	7	14	27	35	9	21	31
Nostocophycideae ; Nostocaceae	0	1	0	1	0	1	33	0	0	2	0	1	0	0	2
Bacilli ; Bacillaceae	2	3	1	13	43	23	34	4	3	5	9	39	4	4	6
Clostridia ; Lachnospiraceae	3	11	2	12	28	0	11	2	0	6	21	15	89	4	13
Clostridia ; Ruminococcaceae	2	11	2	4	9	3	27	0	2	4	19	15	280	3	7
Planctomycea ; Planctomycetaceae	16	16	6	11	17	8	9	9	12	0	13	21	9	17	6
Alphaproteobacteria ; Bradyrhizobiaceae	3	2	1	9	73	1	1	1	9	0	6	37	6	19	0
Alphaproteobacteria ; Rhizobiaceae	0	3	0	14	15	0	3	0	2	27	6	0	0	1	9
Alphaproteobacteria ; Rhodospirillaceae	11	61	4	38	43	13	33	6	13	23	39	57	9	35	52
Alphaproteobacteria ; Sphingomonadaceae	2	30	0	86	52	3	23	18	7	60	33	55	3	6	70
Betaproteobacteria ; Aquabacteriaceae	73	203	75	241	254	143	210	185	124	240	209	220	194	162	215
Betaproteobacteria ; Burkholderiaceae	8	39	4	44	36	3	34	4	22	49	34	50	11	27	56
Betaproteobacteria ; Comamonadaceae	13	109	16	287	100	22	170	117	51	196	191	251	243	53	250
Betaproteobacteria ; Oxalobacteraceae	12	62	11	108	43	27	41	22	41	104	68	83	38	35	77
Betaproteobacteria ; Rhodocyclaceae	4	17	0	20	10	2	27	11	11	25	30	36	25	15	33
Gammaproteobacteria ; Aeromonadaceae	1	25	3	38	17	0	51	0	2	44	36	32	0	2	18
Gammaproteobacteria ; Enterobacteriaceae	2	94	17	269	49	31	142	61	32	422	108	210	207	28	159
Gammaproteobacteria ; Pseudomonadaceae	5	309	15	502	132	39	370	147	32	590	342	476	124	169	479
Gammaproteobacteria ; Xanthomonadaceae	0	19	0	61	31	3	19	7	2	30	21	55	4	2	59
Verrucomicrobiae ; Verrucomicrobiaceae	0	6	3	1	14	4	4	4	3	1	7	11	84	3	11
ALL BACTERIAL FAMILIES	469	1812	369	2828	1975	625	1926	891	769	2536	2082	2847	2015	1050	2582
Enterococcus (MPN/100mL)	10	275	171	3551	85	410	7701	128	139	2310	98	12997	2481	41	605
E. coli (MPN/100mL)	52	31	62	1695	327	323	11199	598	171	121	122	3076	2489	31	238

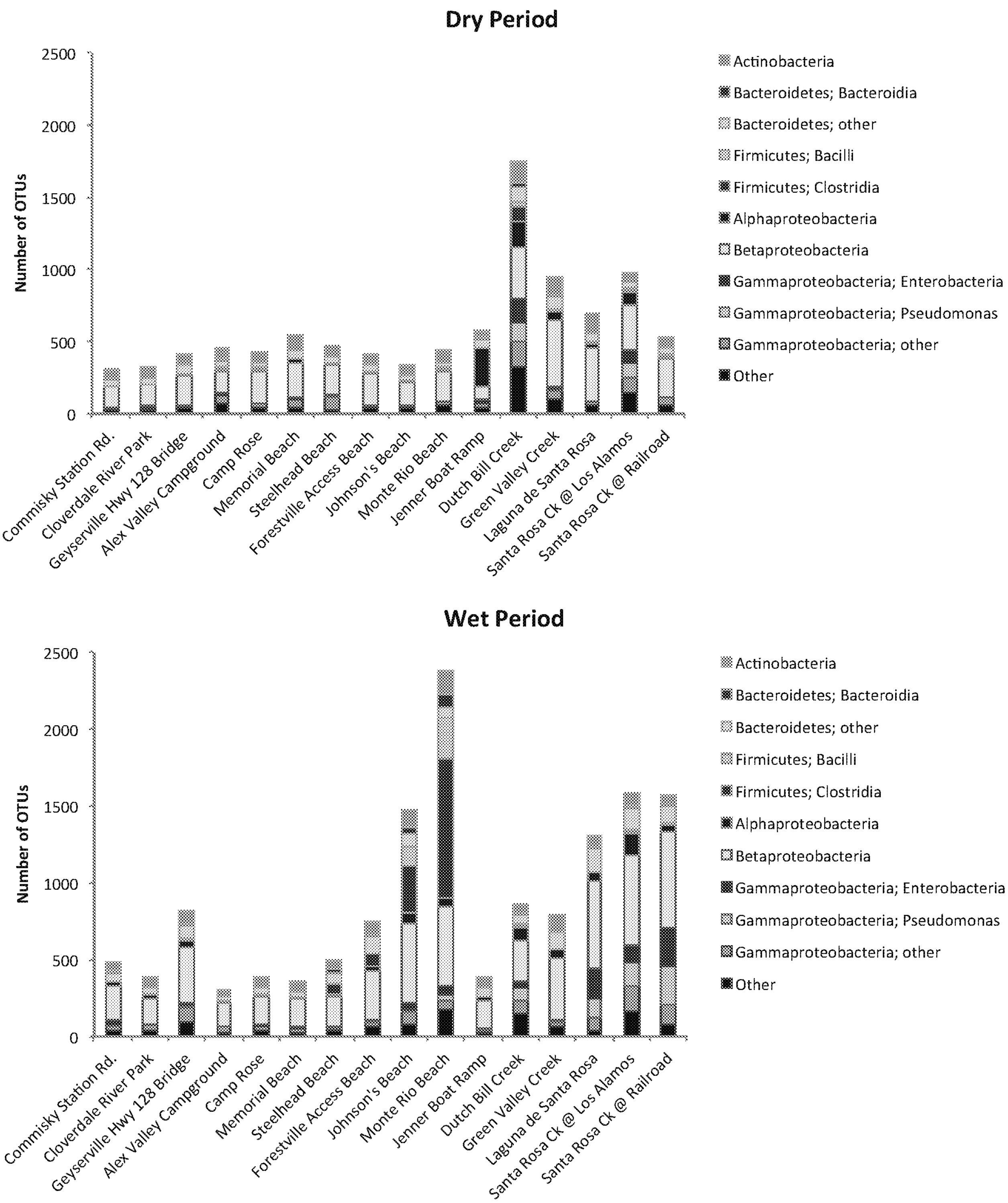


Figure 2-1. Bacterial community composition of dry and wet period samples at Russian River beaches and tributaries in impaired watersheds. Taxonomic richness of the bacterial community is shown as the number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.

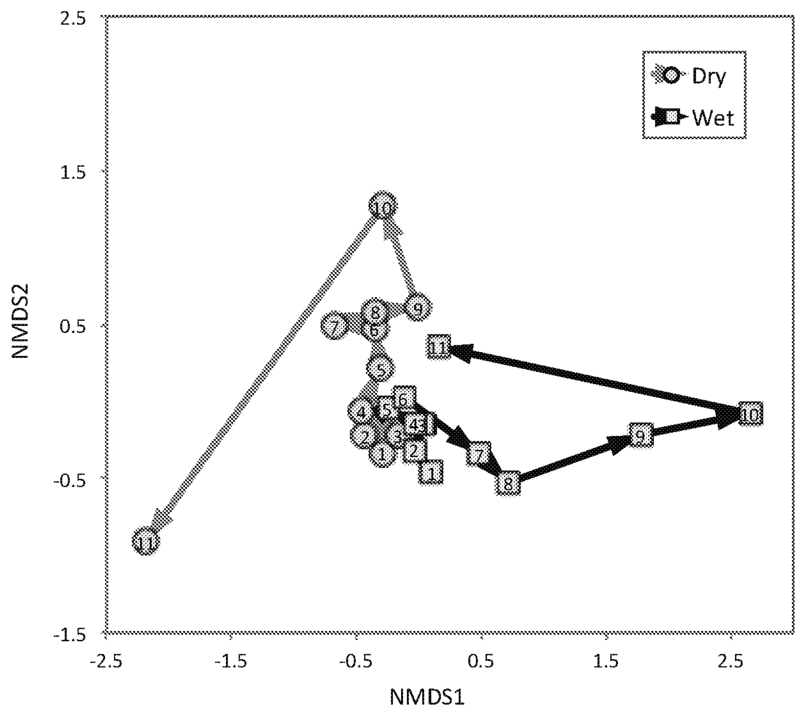


Figure 2-2. Changes in bacterial community structure from upstream to downstream sites along the Russian River during dry and wet periods. Arrows point from upstream to downstream sites and symbols are numbered sequentially from upstream to downstream as follow: (1) Commisky Station Road, (2) Cloverdale River Park, (3) Geyserville Highway 28 Bridge, (4) Alexander Valley Campground, (5) Camp Rose, (6) Memorial Beach, (7) Steelhead Beach, (8) Forestville River Access, (9) Johnson's Beach, (10) Monte Rio Beach, (11) Jenner Boat Ramp. Ordination conducted by NMDS with Bray–Curtis distance metric (2D stress = 0.09).

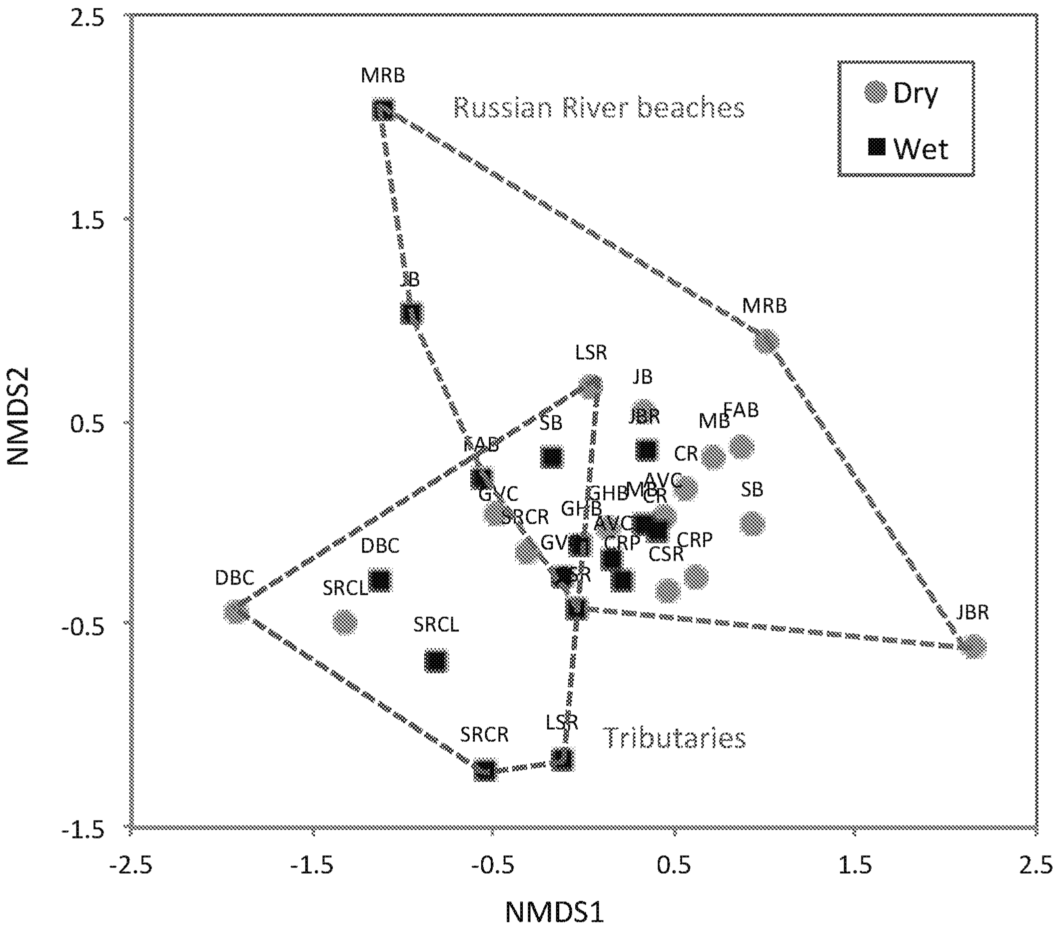


Figure 2-3: Variation in bacterial community structure in Russian River and surrounding watershed samples during dry and wet periods. Ordination conducted by NMDS with Bray–Curtis distance metric (2D stress = 0.13). Labels are location codes (Table 1).

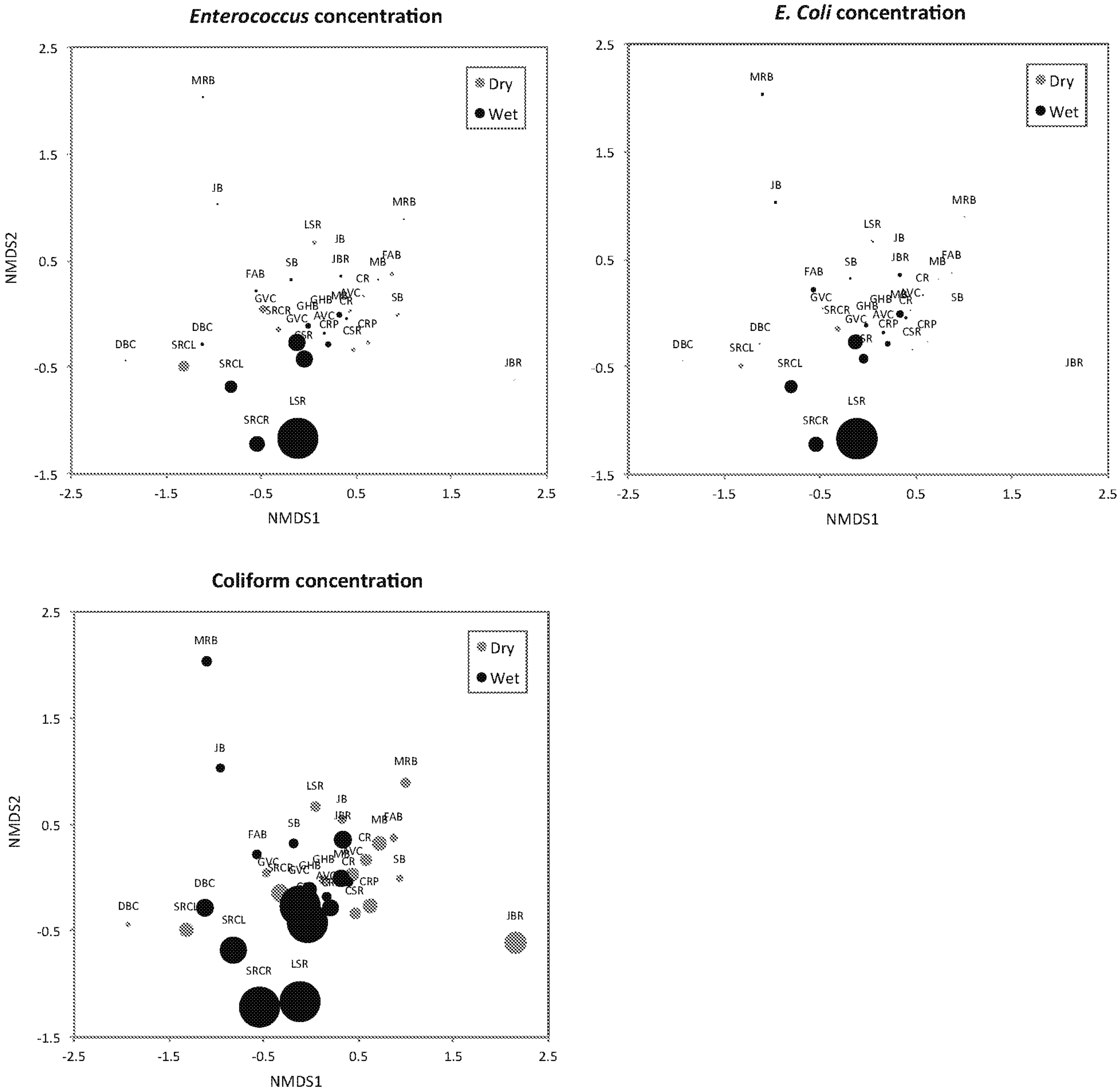


Figure 2-4: Relationship between bacteria community structure and concentrations of fecal indicator bacteria. NMDS ordination configurations are identical to Figure 2-2 but symbol areas are scaled to maximum *Enterococcus*, *E. coli* and coliform concentrations (MPN/100 mL) measured by conventional fecal indicator tests.

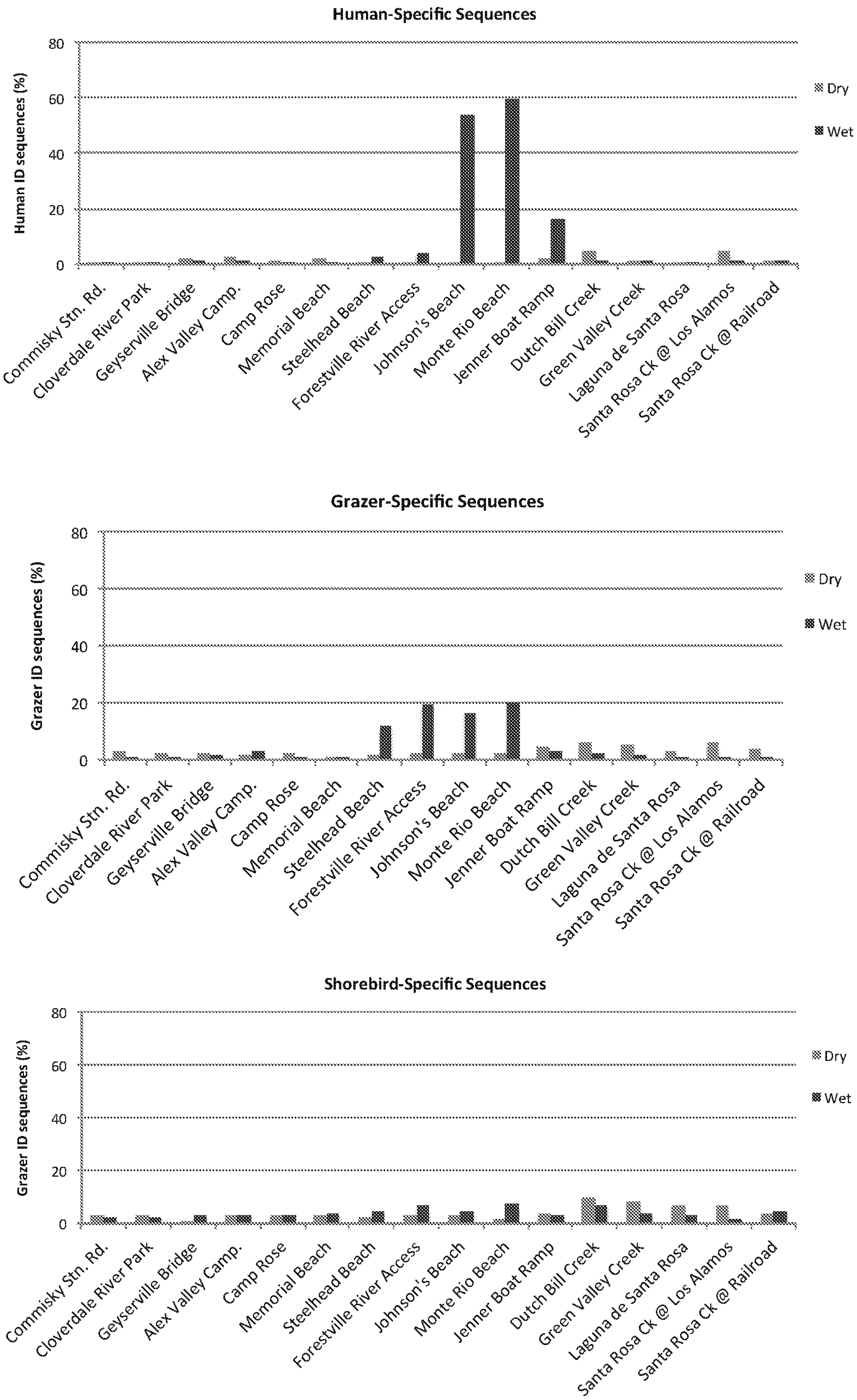
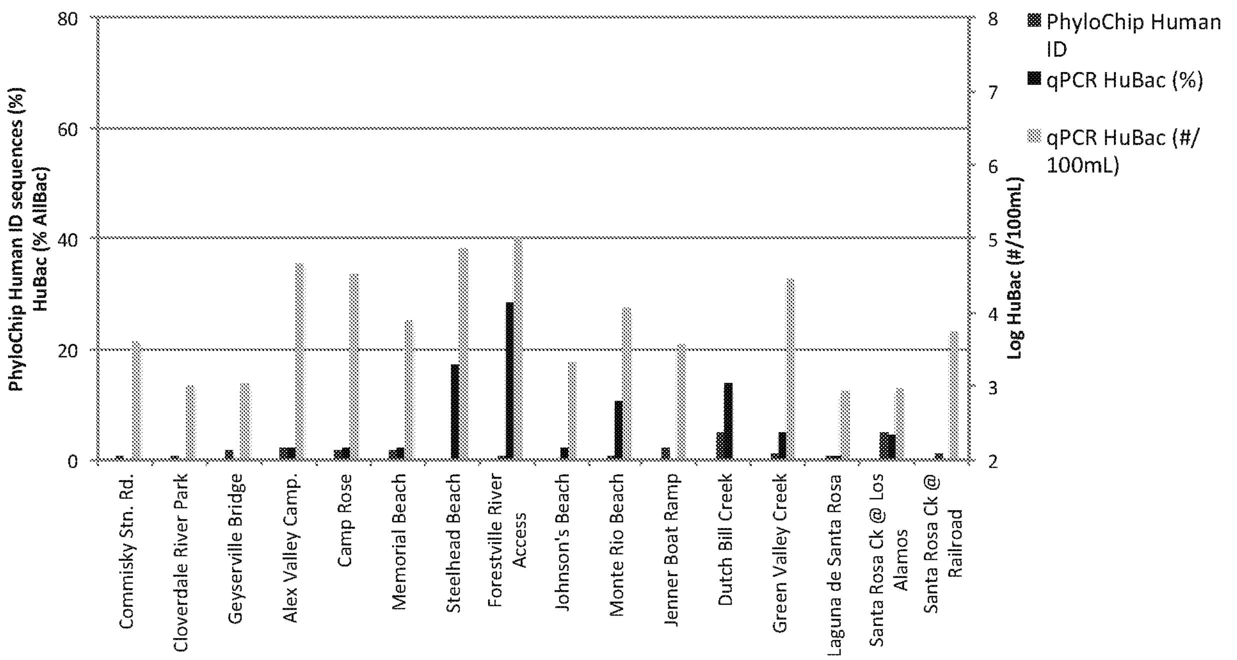


Figure 2-5. Fecal source detection results during dry and wet periods. Values are the percent of source-specific 16S rRNA gene targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.

Dry Period - Human Markers



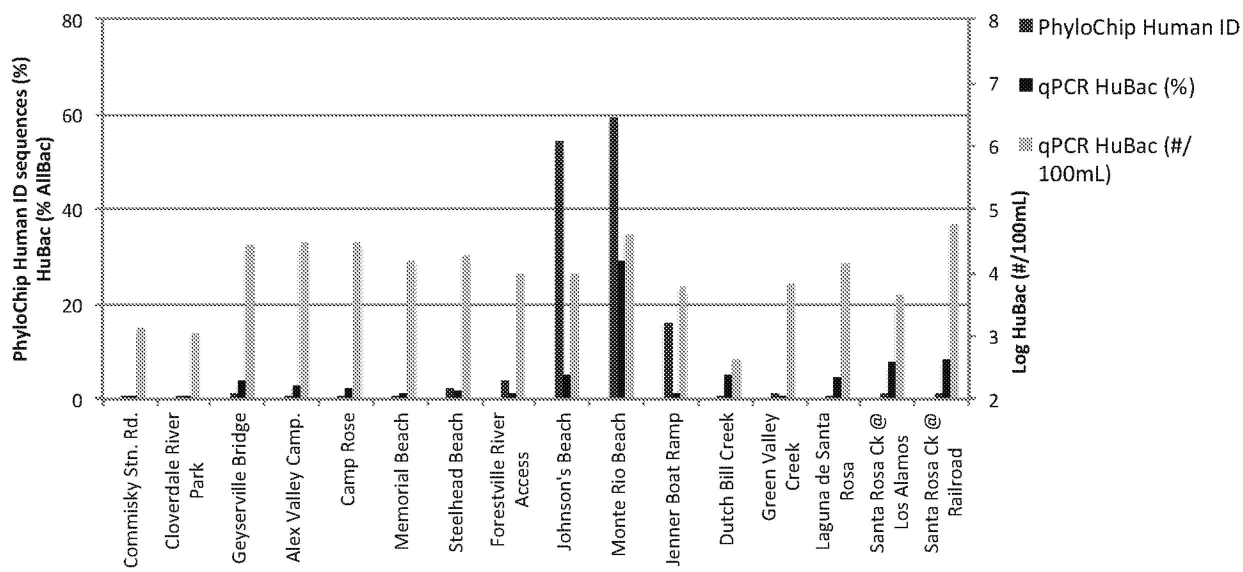


Figure 2-6. Comparison of PhyloChip human fecal ID results from Task 1 to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.

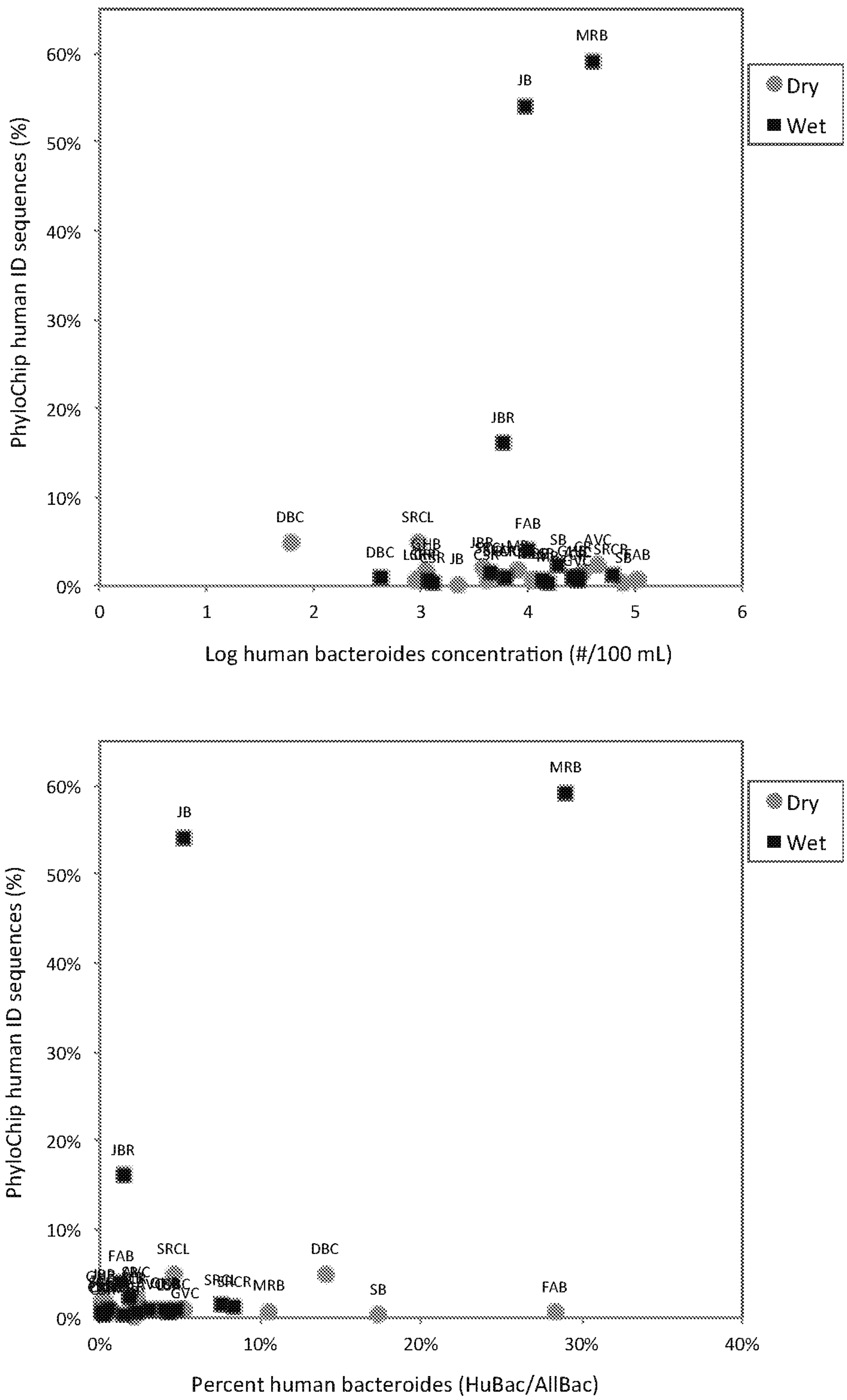


Figure 2-7. Relationship between PhyloChip human fecal ID results from Task 1 to qPCR estimates of human *Bacteroides* concentration (a) and human *Bacteroides* relative abundance (b). Correlation r=0.13 and r=0.42 for PhyloChip human ID % vs human *Bacteroides* concentration (a) and relative abundance (b), respectively.

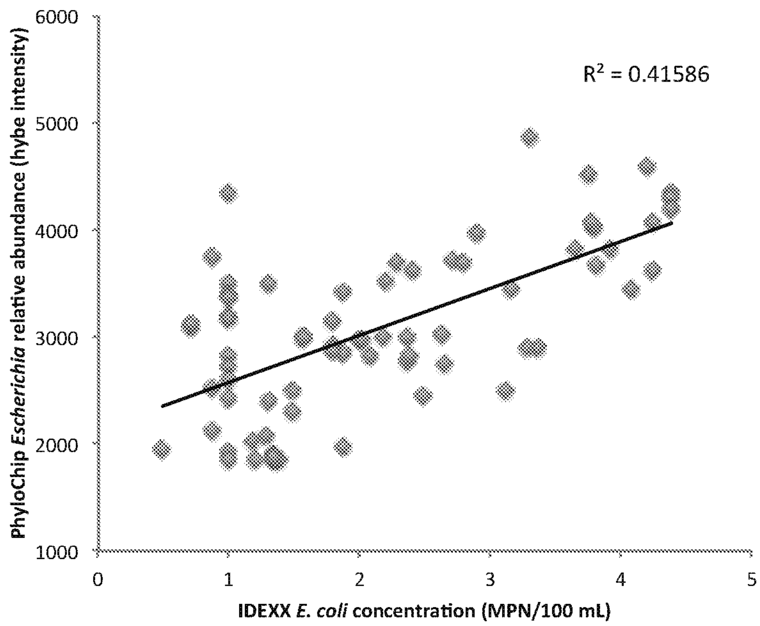
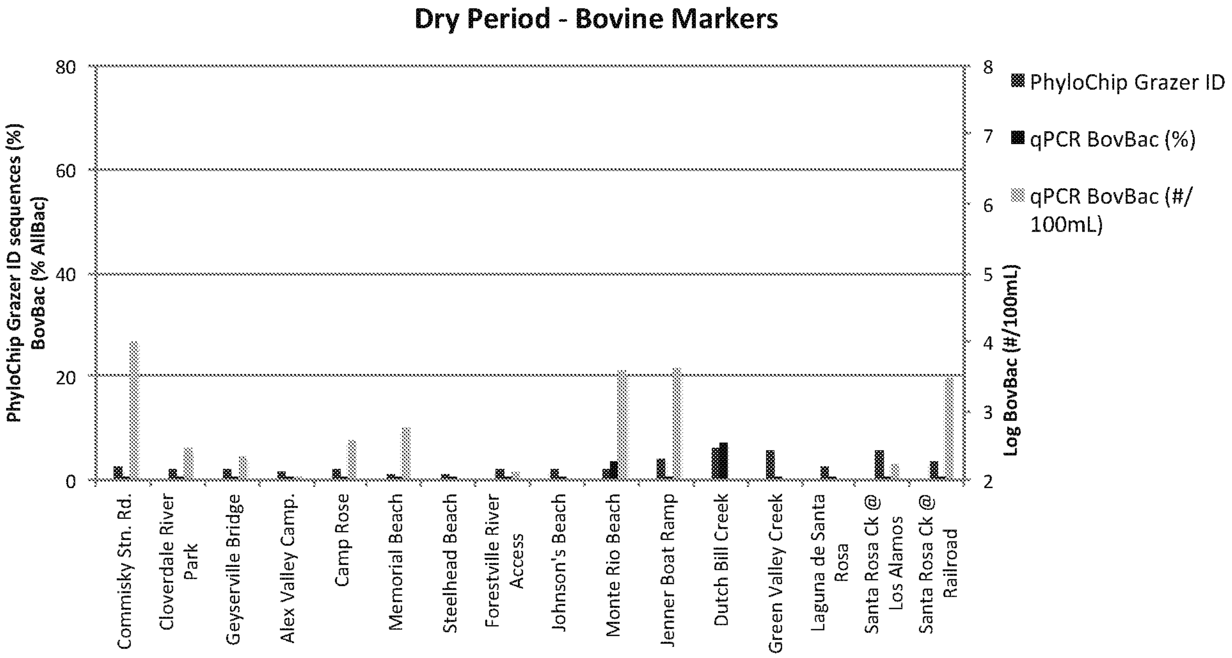


Figure 2-8. Relationship between IDEXX *E. coli* concentration and PhyloChip *Escherichia* relative abundance measured as the mean hybridization intensity of all detected *Escherichia* OTUs.



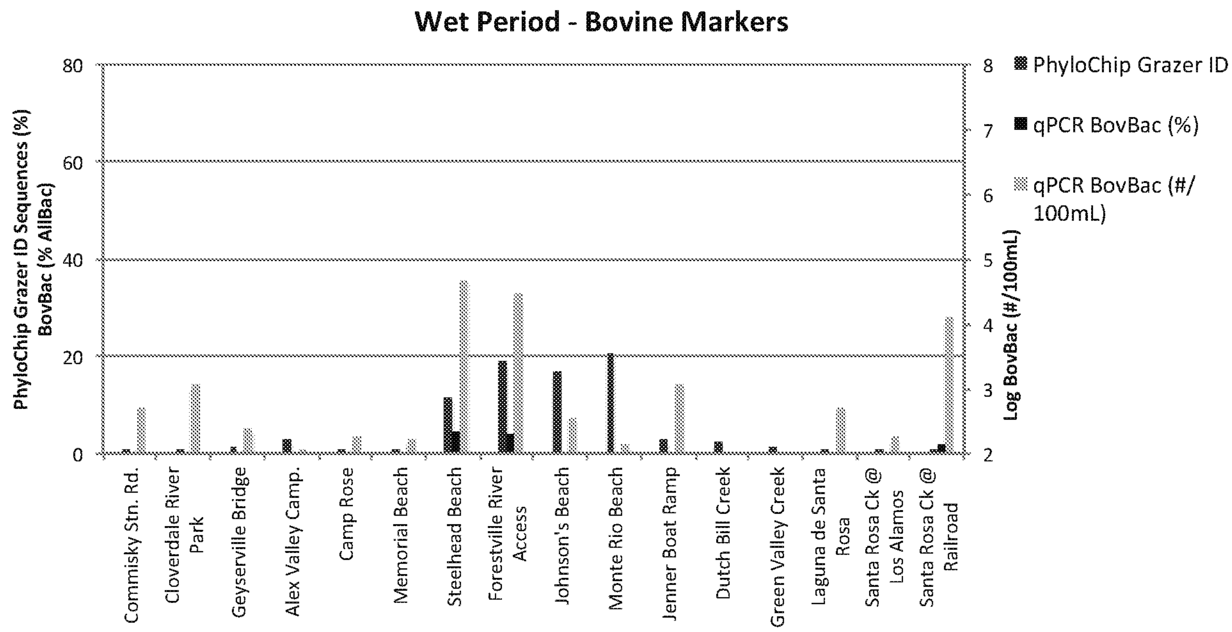
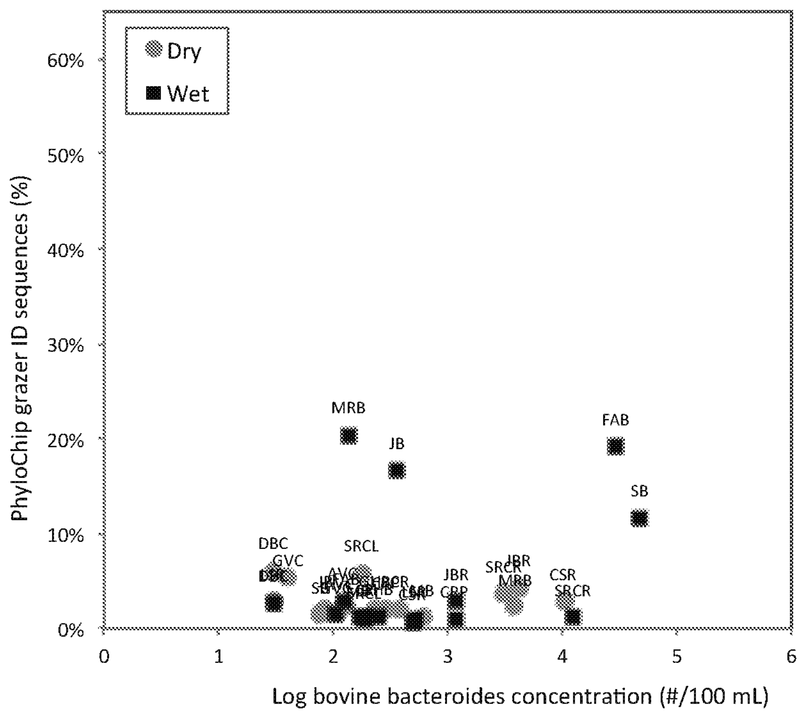


Figure 2-9. Comparison of PhyloChip grazing mammal fecal ID results from Task 1 to qPCR estimates of bovine *Bacteroides* as measured by the BovBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 721 grazer-specific sequences targeted by the test. BovBac results are reported as both estimates of concentration (#/100mL) and relative abundance to total *Bacteroides* as measured by the AllBac qPCR test.



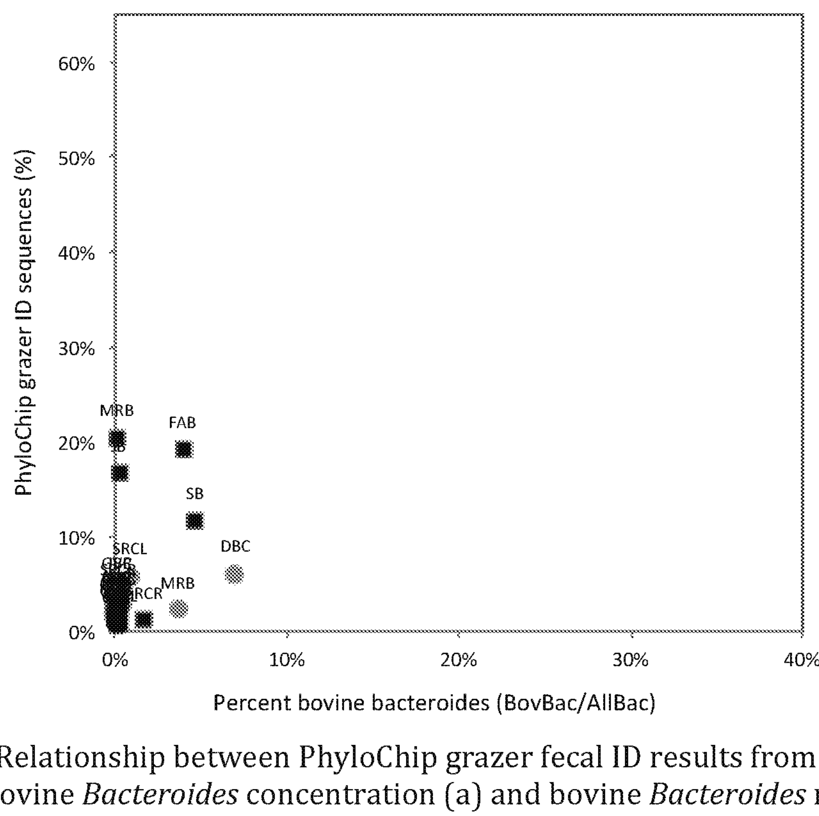


Figure 2-10. Relationship between PhyloChip grazer fecal ID results from Task 1 to qPCR estimates of bovine *Bacteroides* concentration (a) and bovine *Bacteroides* relative abundance (b). Correlation r=0.25 and r=0.35 for PhyloChip grazer ID % vs bovine *Bacteroides* concentration (a) and relative abundance (b), respectively.

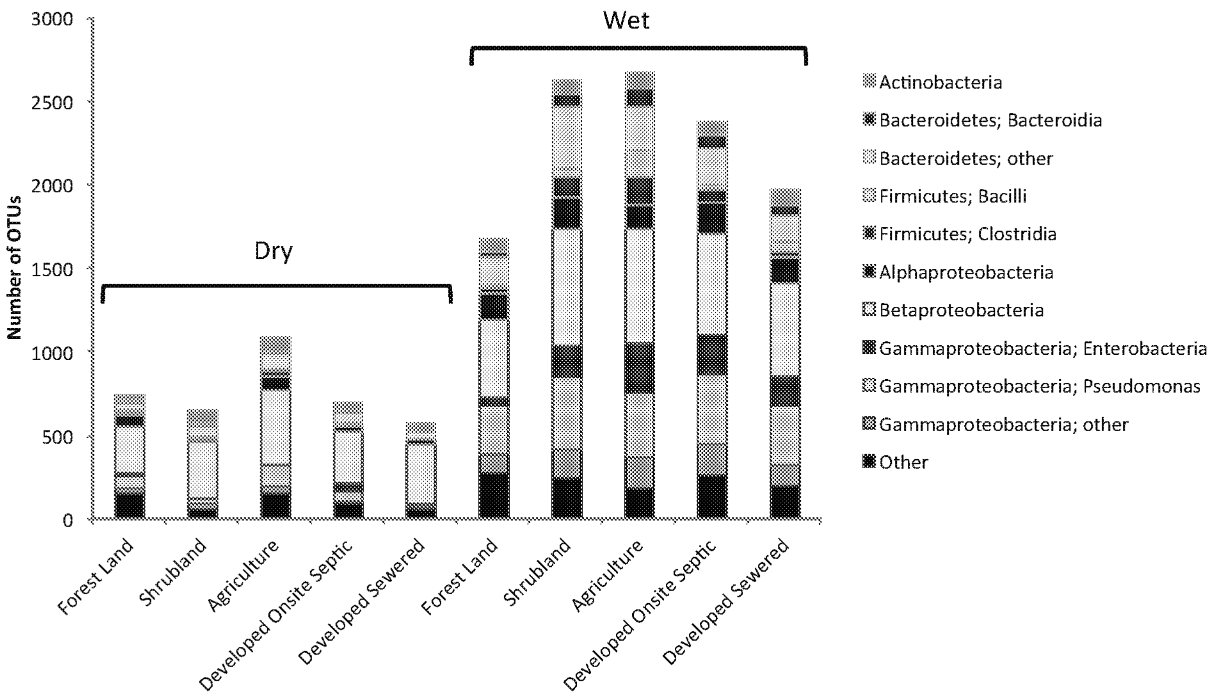


Figure 3-1. Bacterial community composition in different land use types during dry and wet periods. Taxonomic richness of the bacterial community is shown as the median number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.

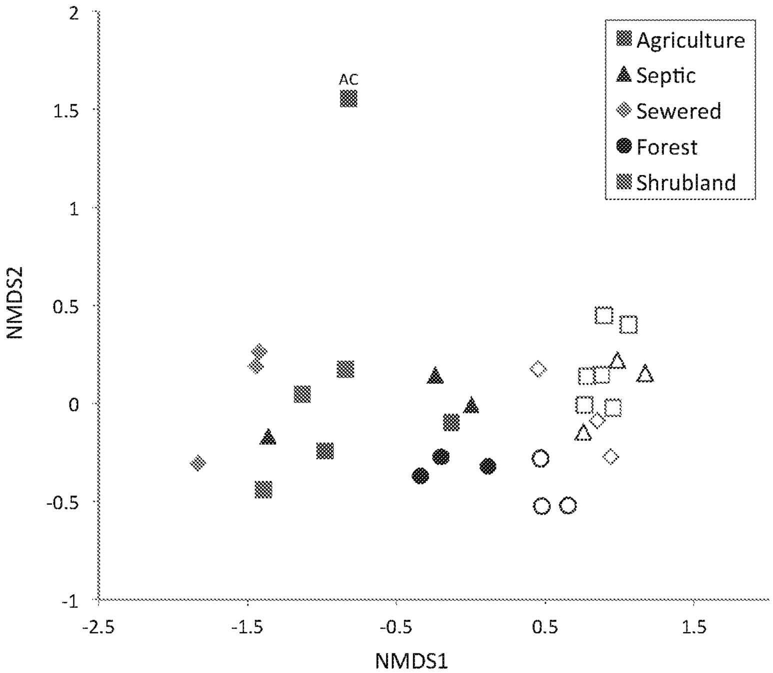


Figure 3-2: Variation in bacterial community structure in different land use types during dry (closed symbols) and wet (open symbols) periods. Ordination conducted by NMDS with Bray–Curtis distance metric (2D stress = 0.06). The data point labeled AC is Abramson Creek.

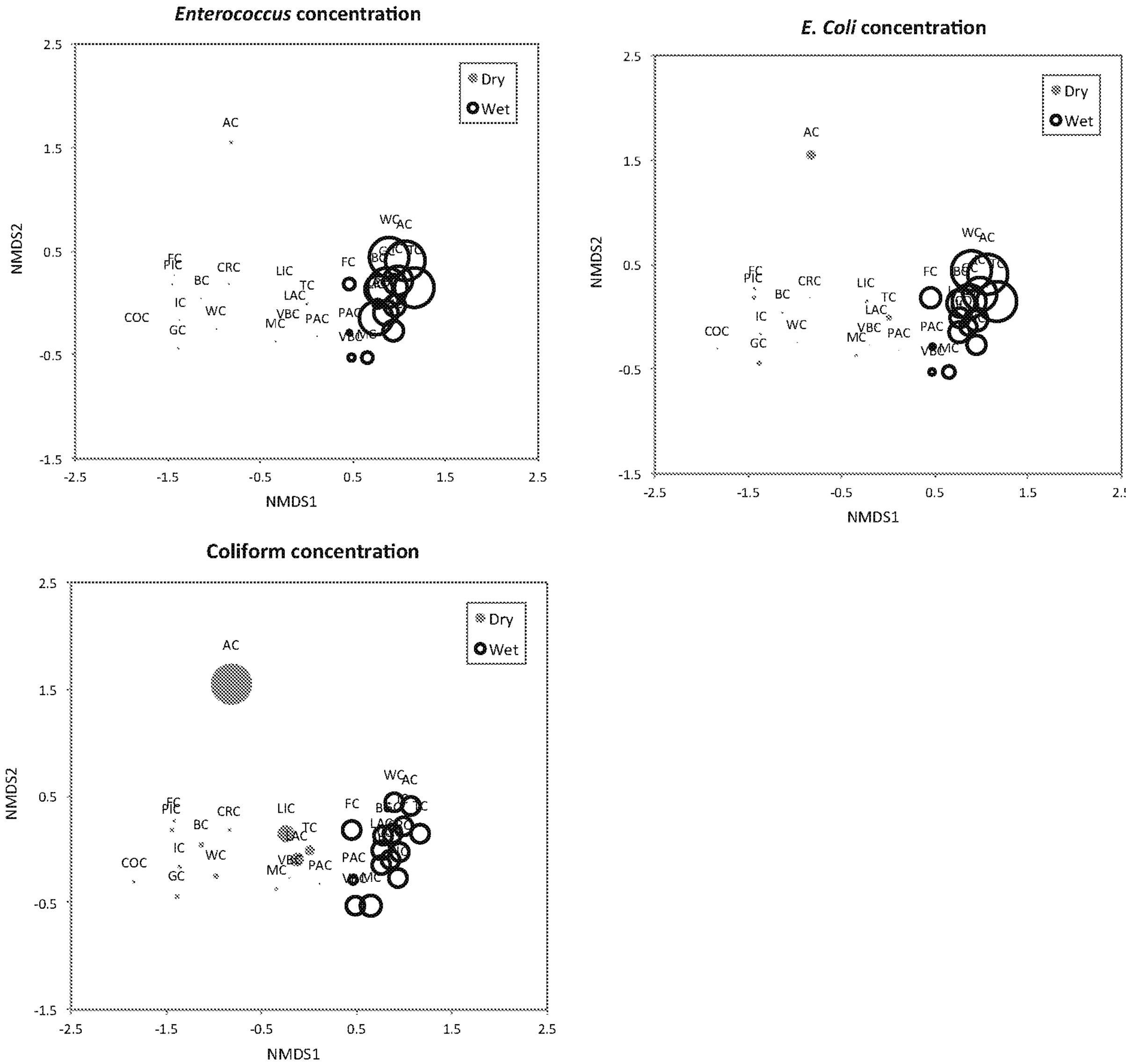


Figure 3-3: Relationship between bacteria community structure and concentrations of fecal indicator bacteria. NMDS ordination configurations are identical to Figure 3-2 but symbol areas are scaled to maximum *Enterococcus, E. coli* and coliform concentrations (MPN/100 mL) measured by conventional fecal indicator tests. The data point labeled AC is Abramson Creek.

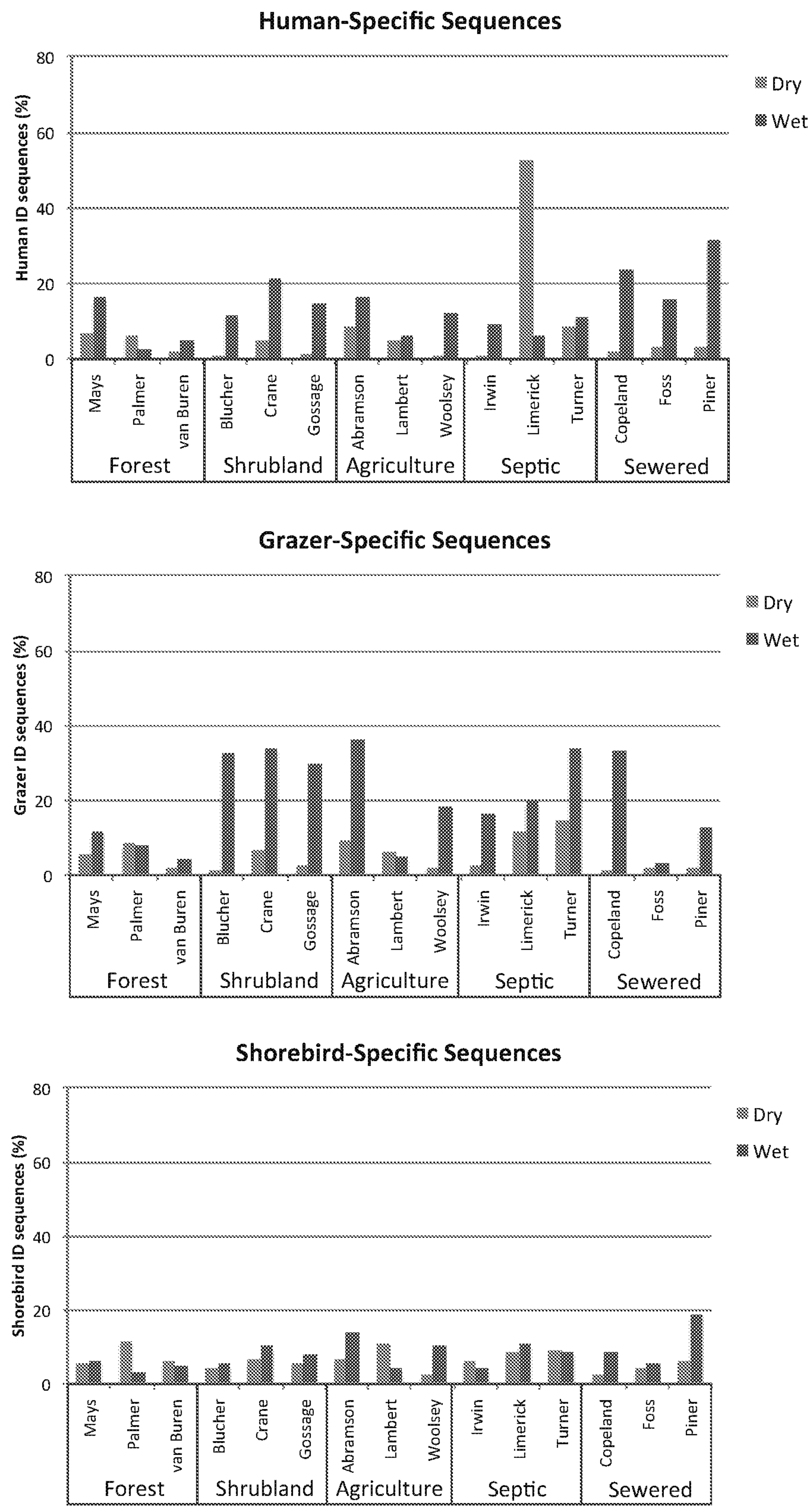
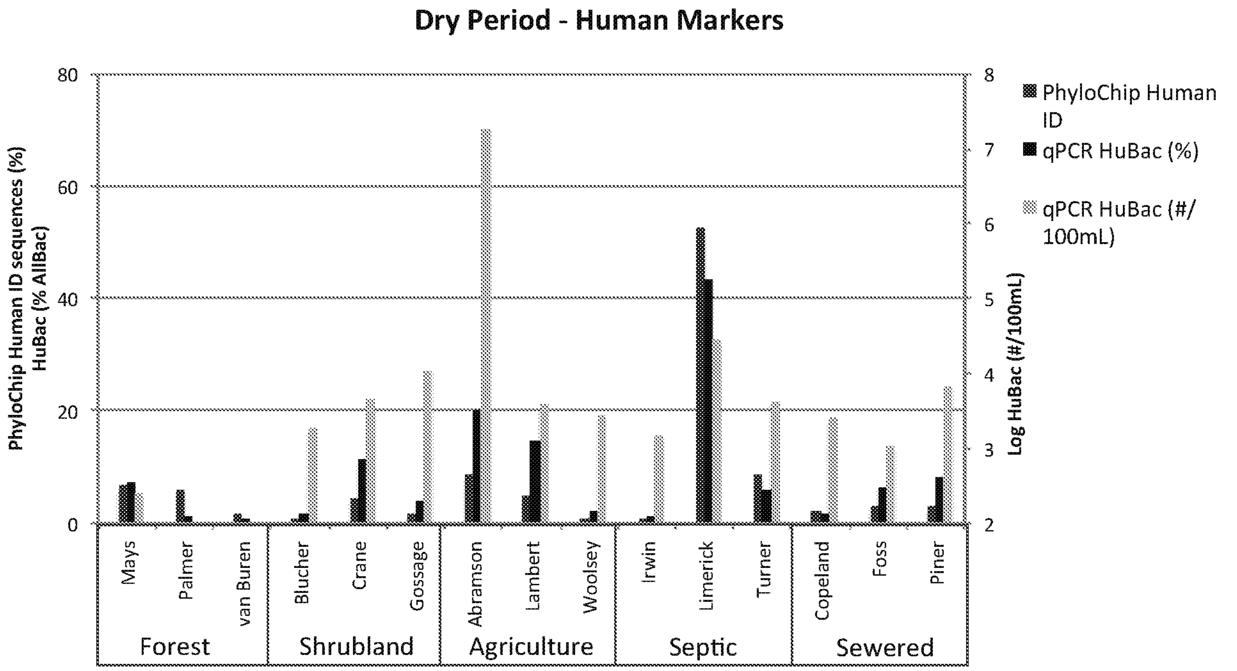


Figure 3-4. Fecal source detection results during dry and wet periods in different land use areas. Values are the percent of source-specific 16S rRNA gene targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.



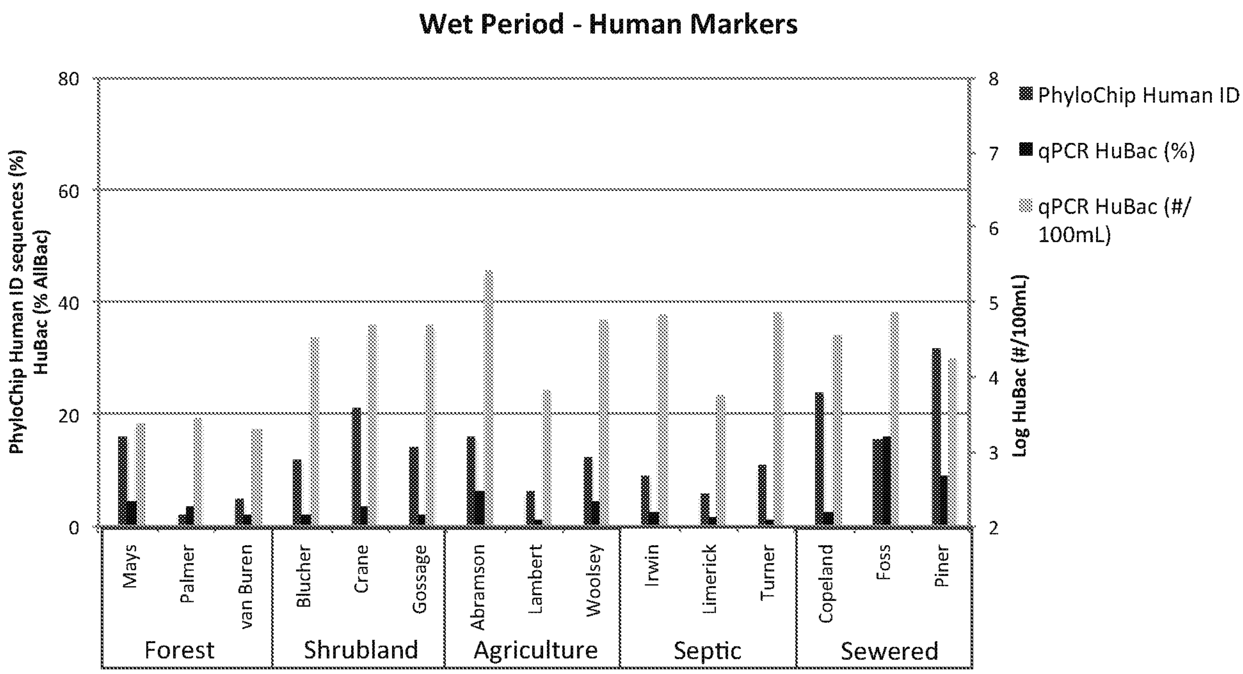


Figure 3-5. Comparison of PhyloChip human fecal ID results from Task 2 to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.

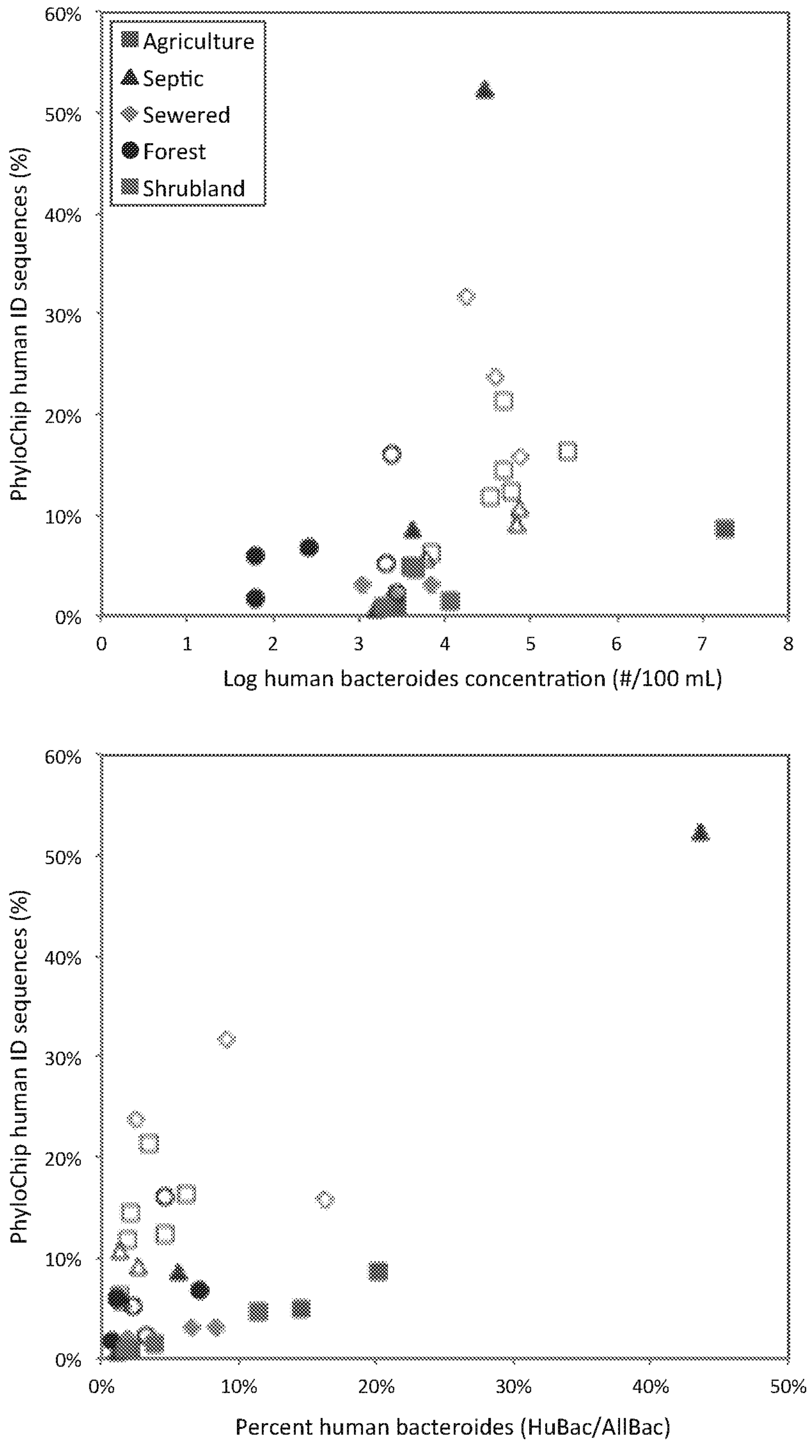


Figure 3-6. Relationship between PhyloChip human fecal ID results from Task 2 to qPCR estimates of human *Bacteroides* concentration (a) and human *Bacteroides* relative abundance (b). Correlation r=0.38 and r=0.66 for PhyloChip human ID % vs human *Bacteroides* concentration (a) and relative abundance (b), respectively.

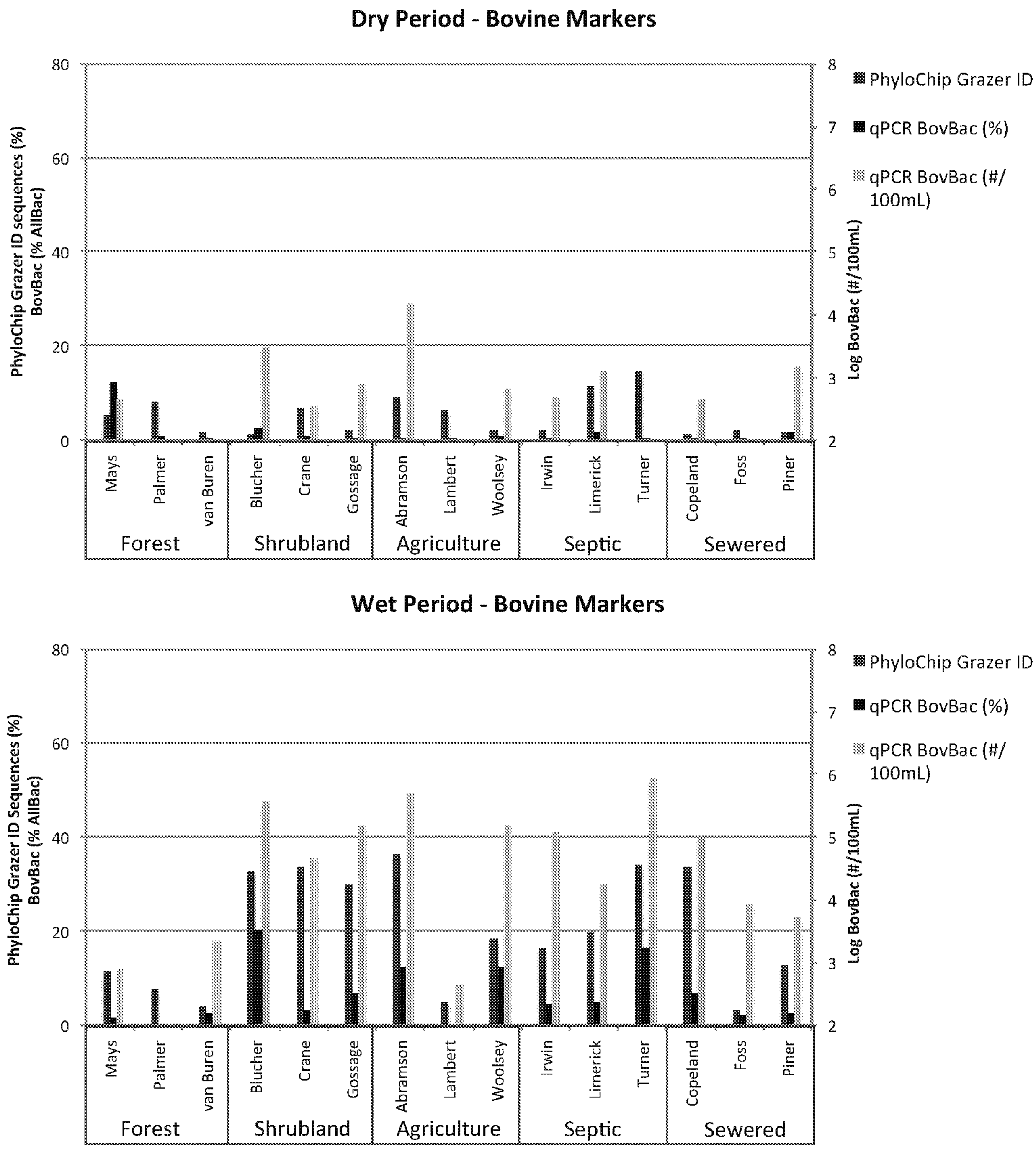
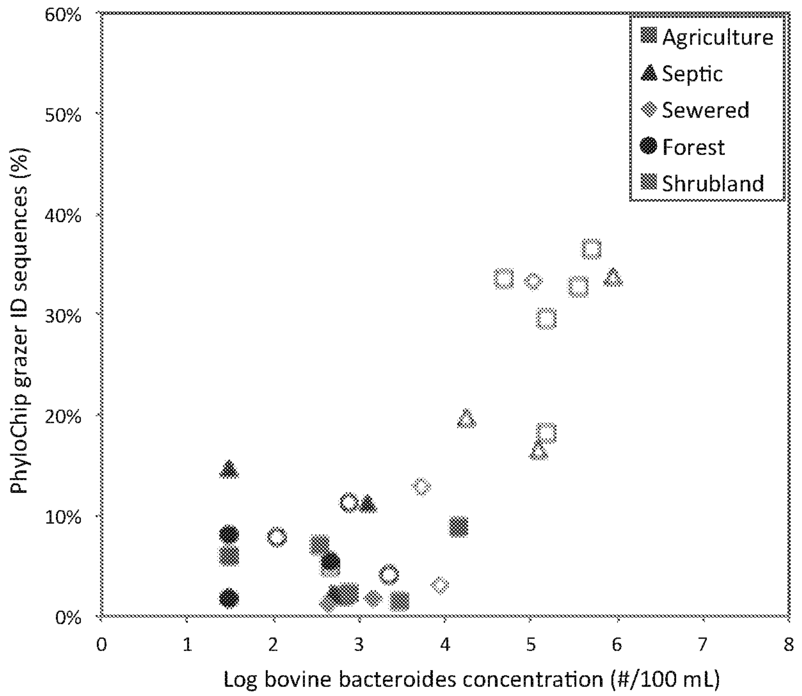


Figure 3-7. Comparison of PhyloChip grazing mammal fecal ID results from Task 2 to qPCR estimates of bovine *Bacteroides* as measured by the BovBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 721 grazer-specific sequences targeted by the test. BovBac results are reported as both estimates of concentration (#/100mL) and relative abundance to total *Bacteroides* as measured by the AllBac qPCR test.



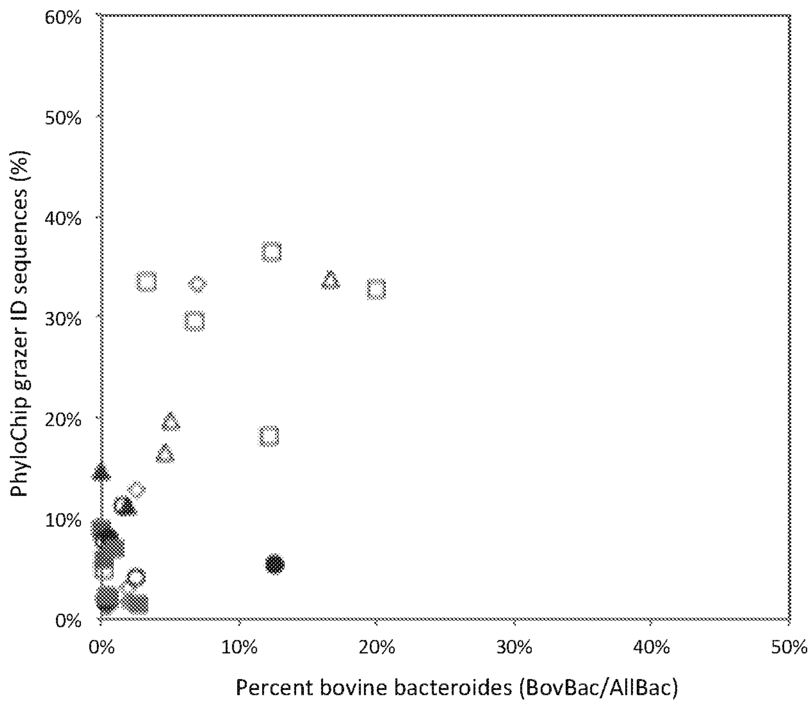
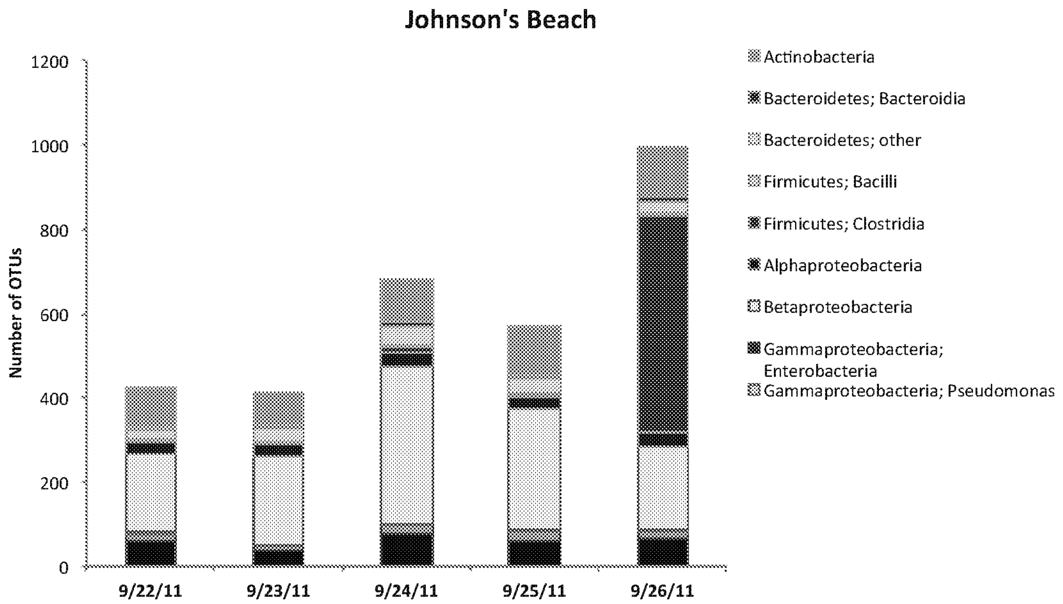


Figure 3-8. Relationship between PhyloChip grazer fecal ID results from Task 2 to qPCR estimates of bovine *Bacteroides* concentration (a) and bovine *Bacteroides* relative abundance (b). Correlation r=0.78 and r=0.70 for PhyloChip grazer ID % vs bovine *Bacteroides* concentration (a) and relative abundance (b), respectively.



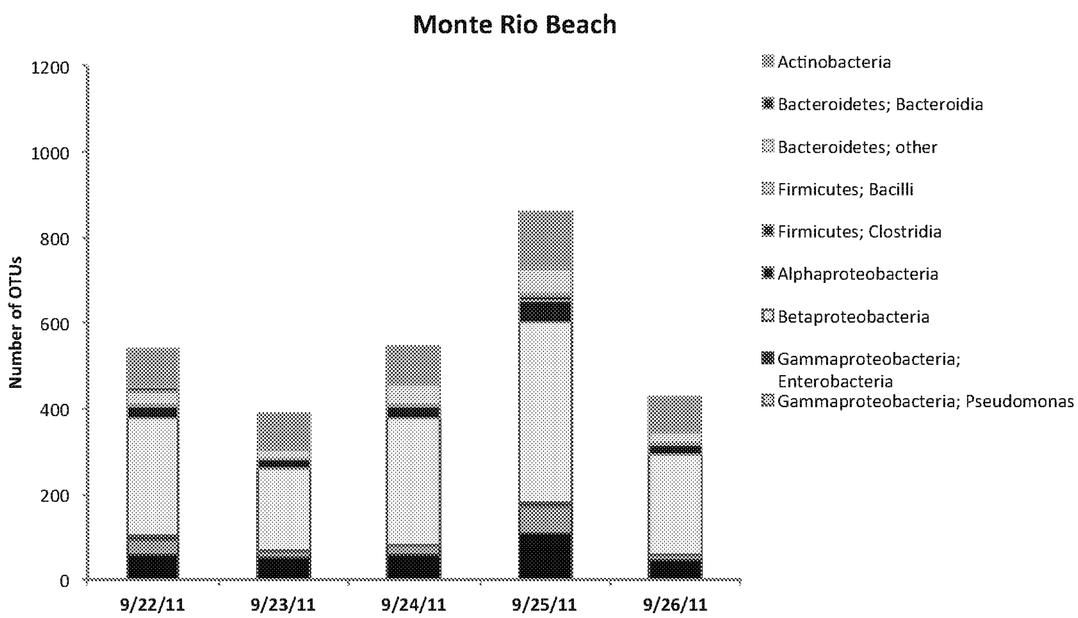


Figure 4-1. Bacterial community composition at Johnson's Beach and Monte Rio Beach during a period of heavy recreational use. Taxonomic richness of the bacterial community is shown as the number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.

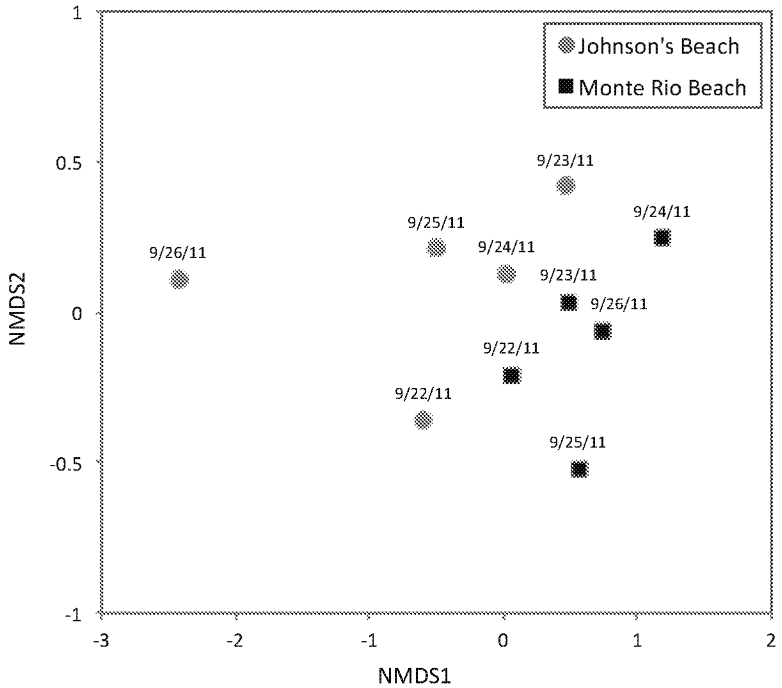


Figure 4-2: Variation in bacterial community structure at Johnson's Beach and Monte Rio Beach during a period of heavy recreational use. Ordination conducted by NMDS with Bray–Curtis distance metric (2D stress = 0.03).

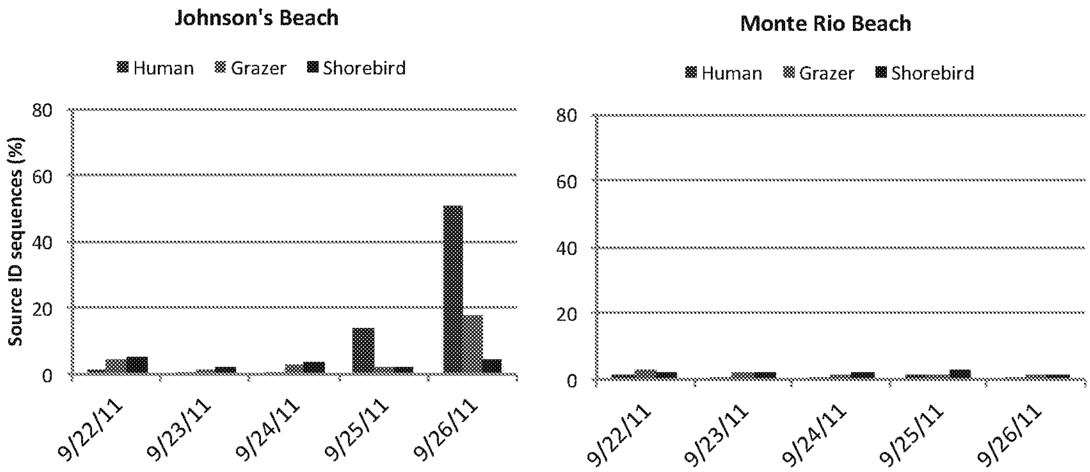


Figure 4-3. Fecal source detection results at Johnson's Beach and Monte Rio Beach. Values are the percent of source-specific 16S rRNA gene sequence targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.

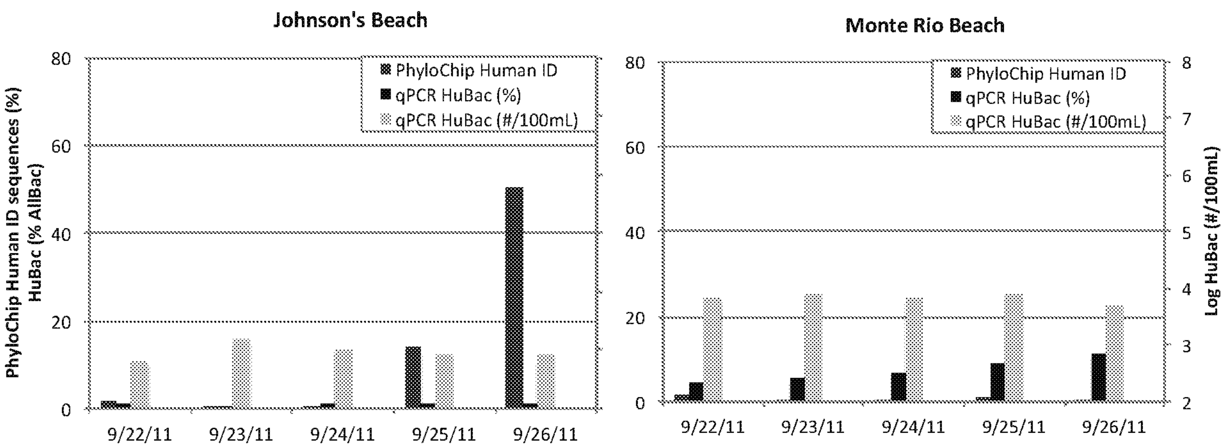


Figure 4-4. Comparison of PhyloChip human fecal ID results from Task 3 to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.

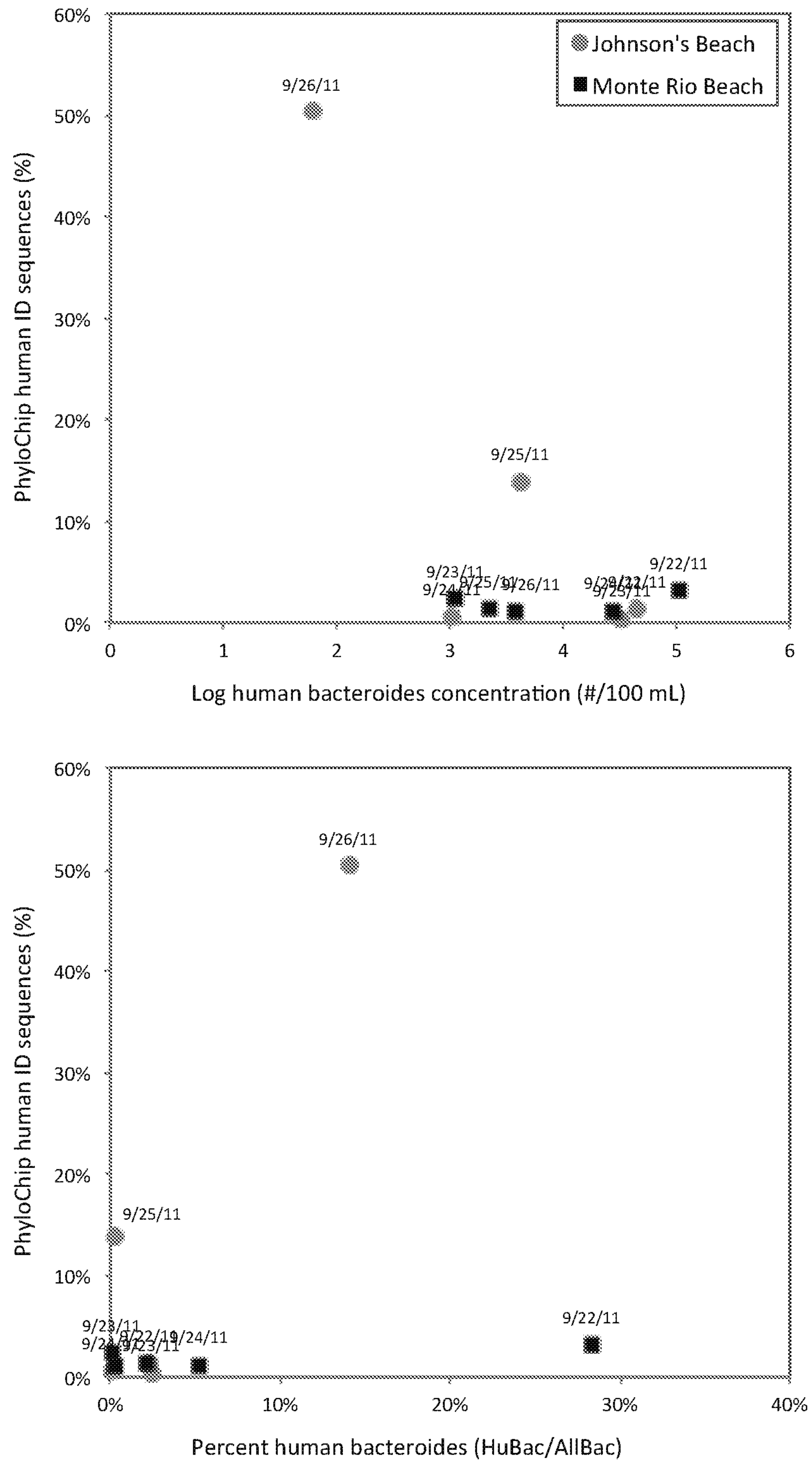


Figure 4-5. Relationship between PhyloChip human fecal ID results from Task 3 to qPCR estimates of human *Bacteroides* concentration (a) and human *Bacteroides* relative abundance (b). Correlation r=-0.68 and r=0.29 for PhyloChip human ID % vs human *Bacteroides* concentration (a) and relative abundance (b), respectively.

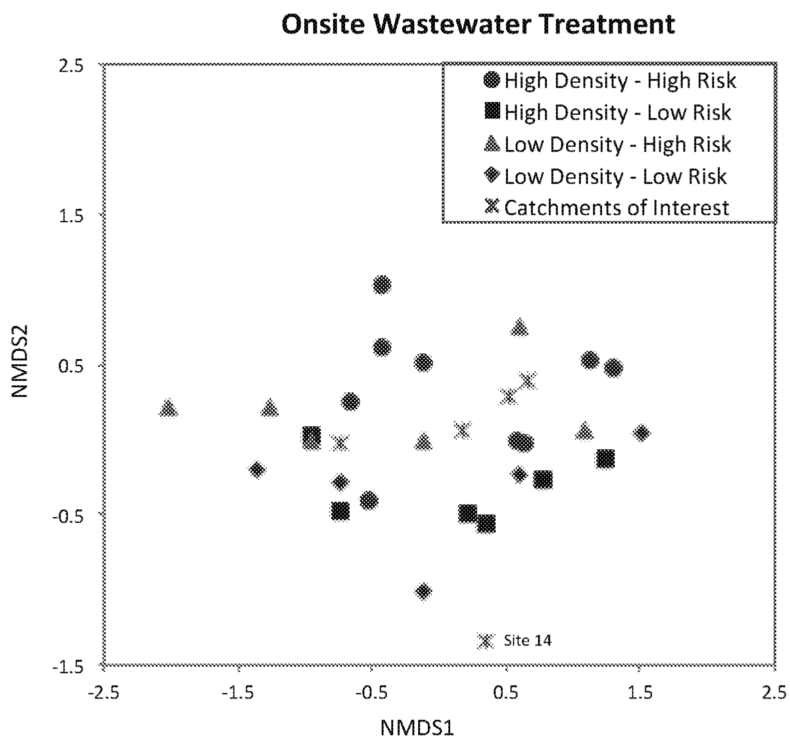


Figure 5-1: Variation in bacterial community structure in high and low parcel density areas with both high and low risk of septic contamination. Ordination conducted by NMDS with Bray–Curtis distance metric (2D stress = 0.13).

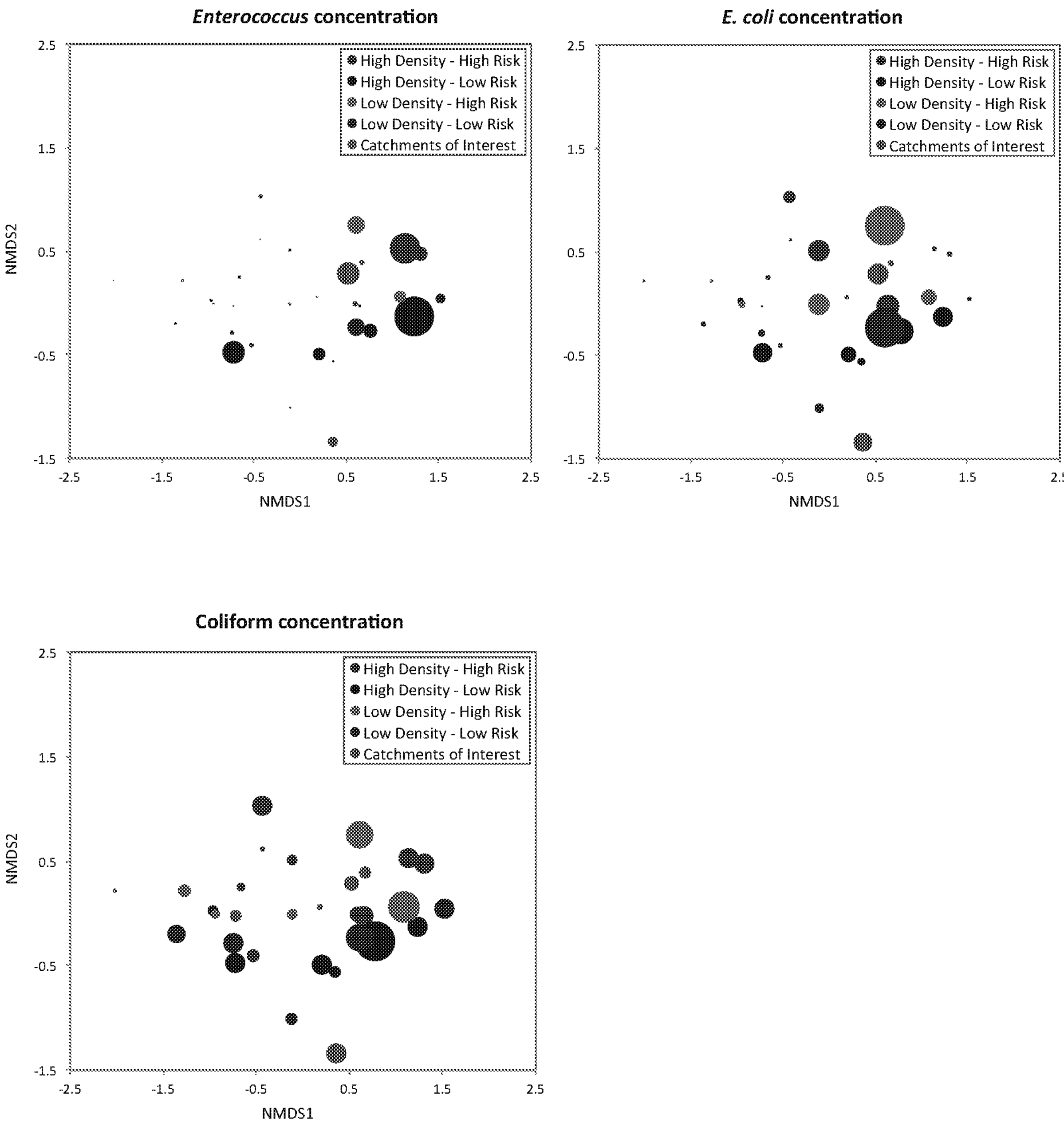
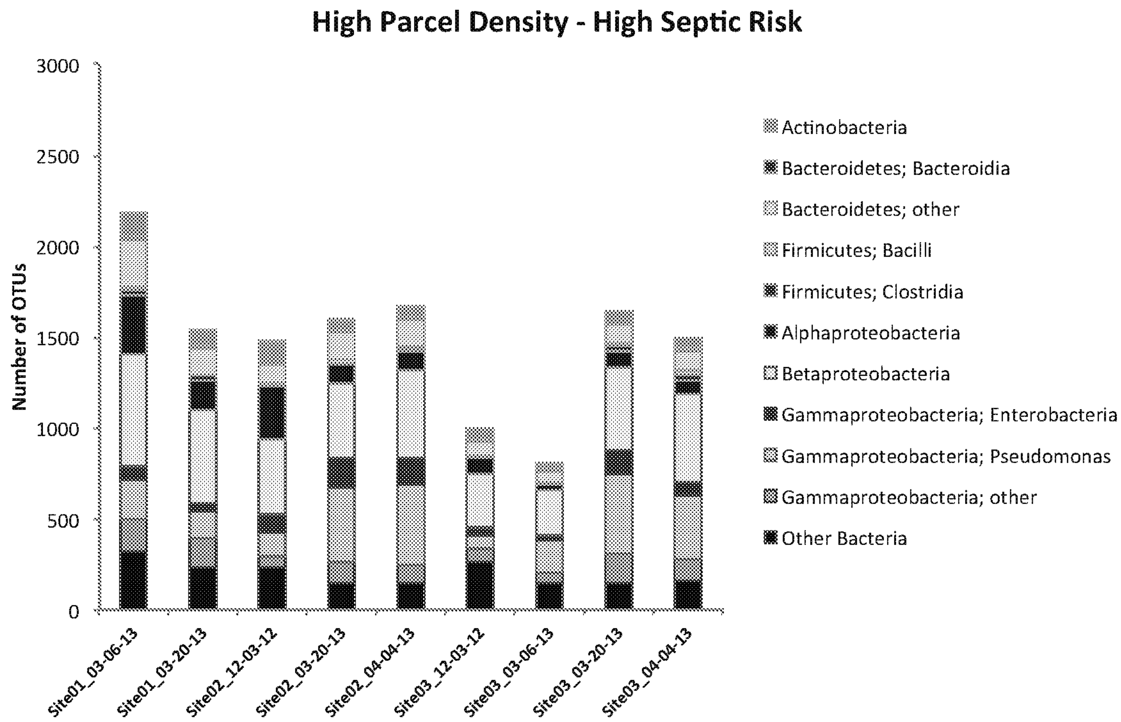


Figure 5-2: Relationship between bacteria community structure and concentrations of fecal indicator bacteria. NMDS ordination configurations are identical to Figure 5-4 but symbol areas are scaled to maximum *Enterococcus*, *E. coli* and coliform concentrations (MPN/100 mL) measured by conventional fecal indicator tests.



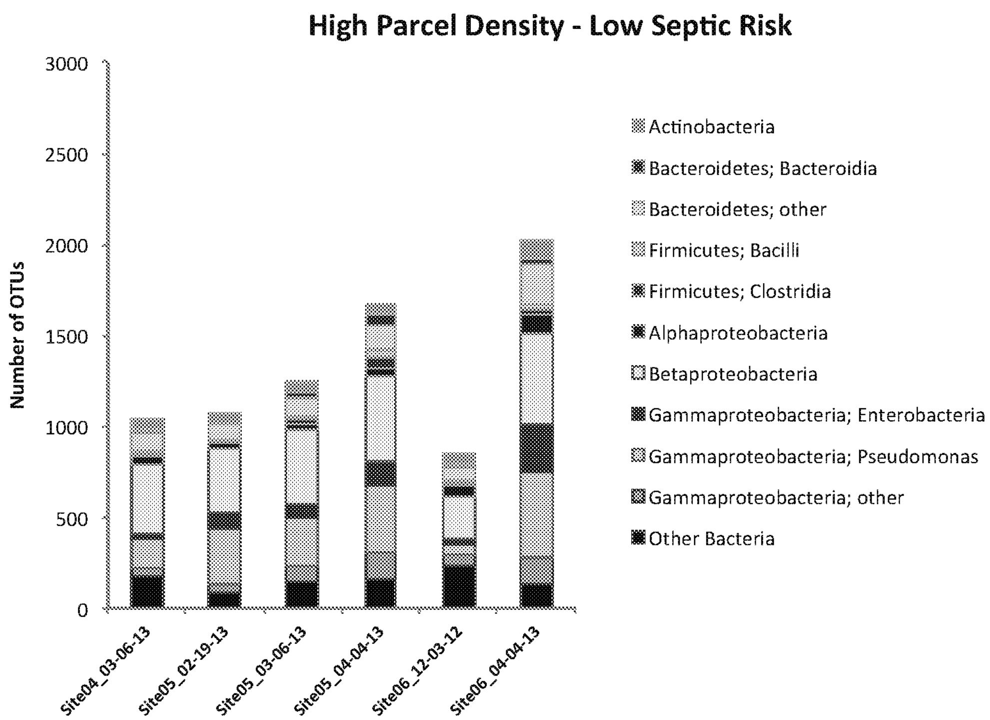


Figure 5-3. Bacterial community composition in high parcel density areas with high and low septic risk. Taxonomic richness of the bacterial community is shown as the number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.

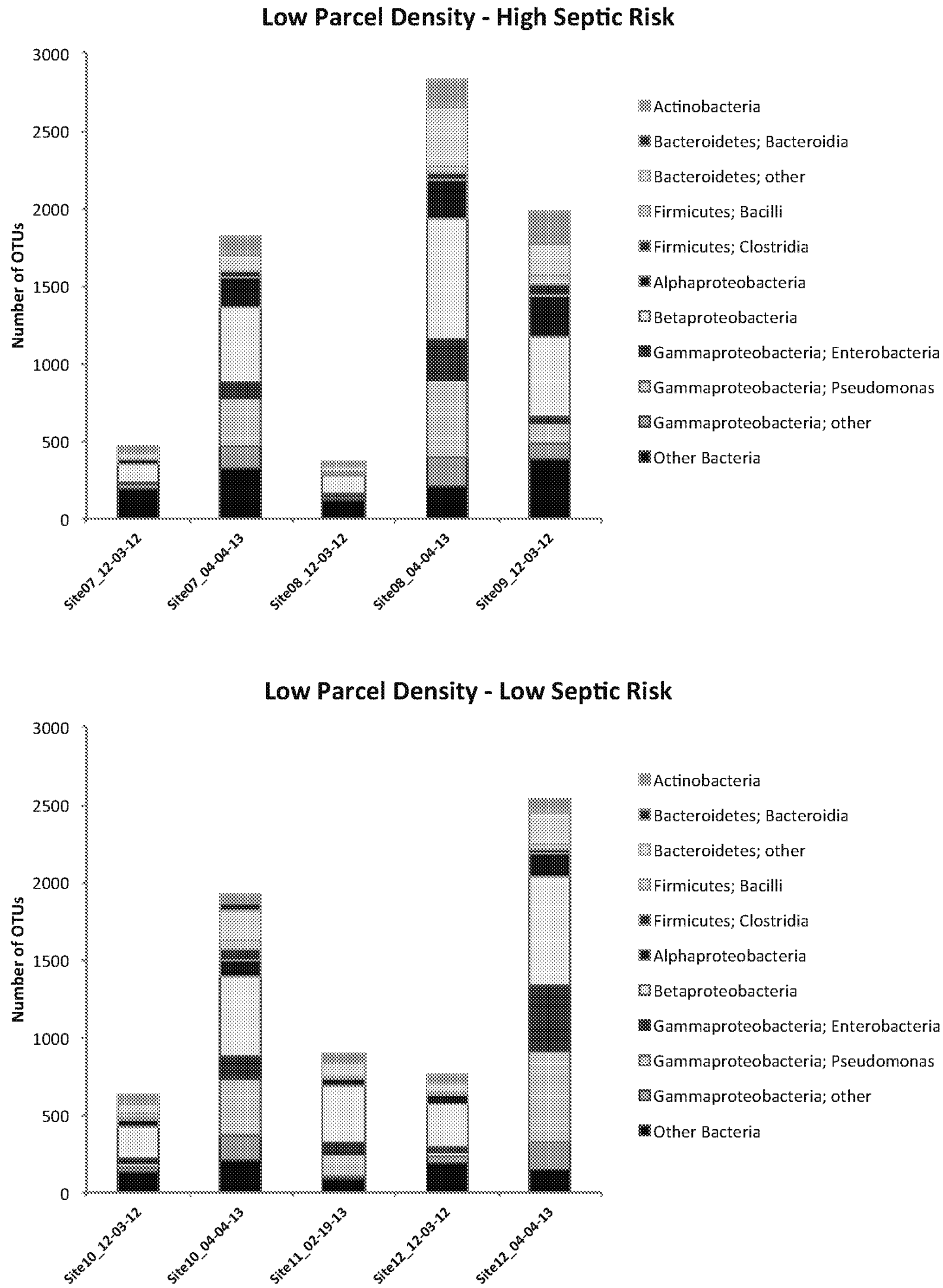


Figure 5-4. Bacterial community composition in low parcel density areas with high and low septic risk. Taxonomic richness of the bacterial community is shown as the number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.

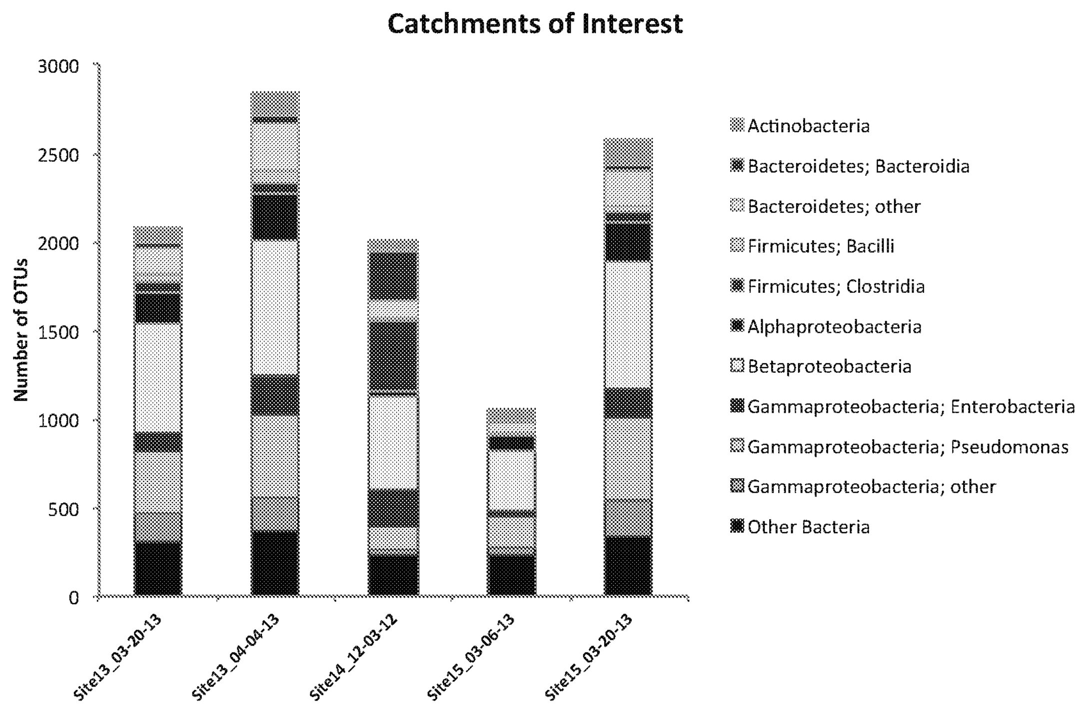
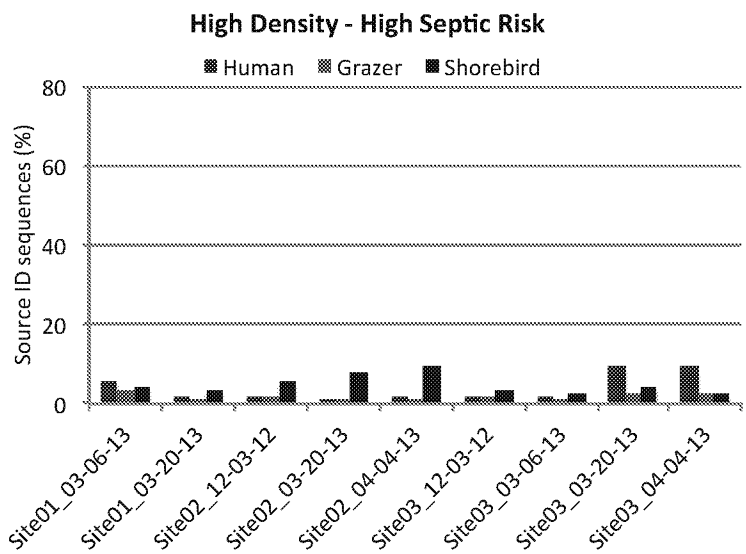


Figure 5-5. Bacterial community composition in catchments of interest (Sites 12-15). Taxonomic richness of the bacterial community is shown as the number of different operational taxonomic units (OTUs) in major bacterial phyla or classes.



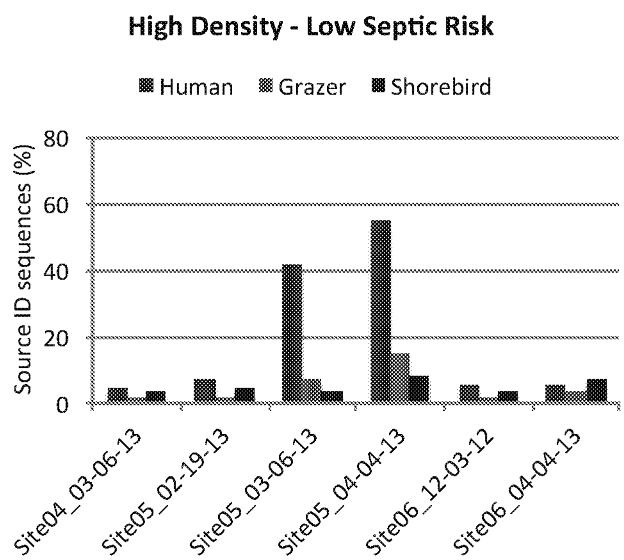


Figure 5-6. Fecal source detection results in high parcel density areas with high and low septic risk. Values are the percent of source-specific 16S rRNA gene sequence targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.

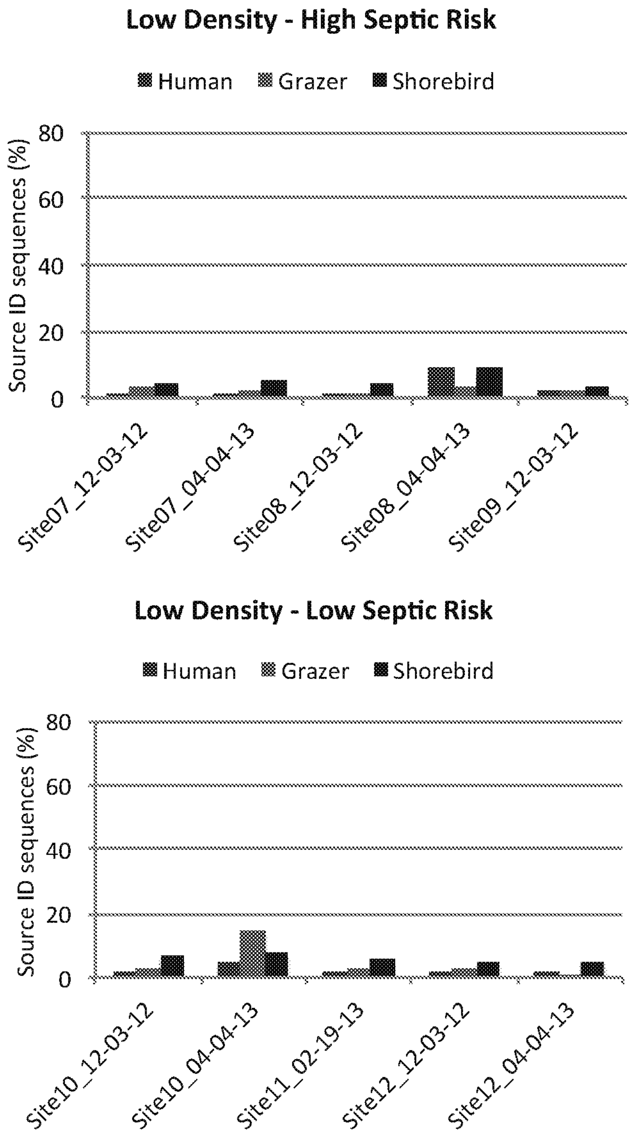


Figure 5-7. Fecal source detection results in low parcel density areas with high and low septic risk. Values are the percent of source-specific 16S rRNA gene sequence targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.

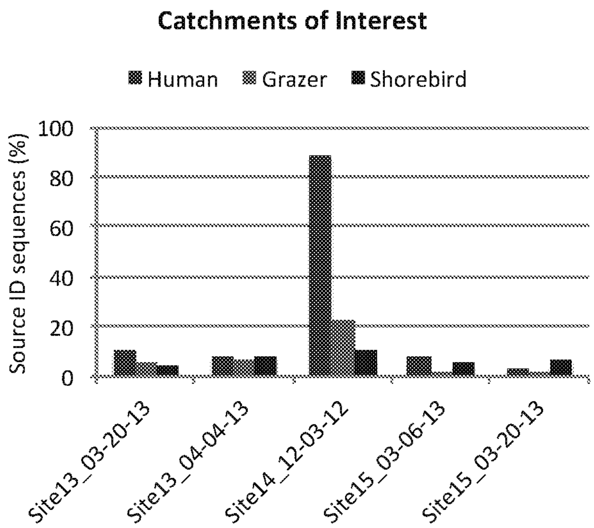
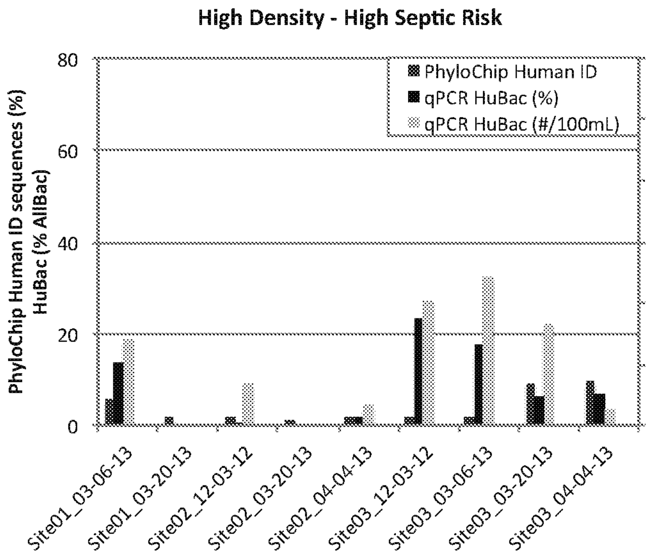


Figure 5-8. Fecal source detection results in catchments of interest. Values are the percent of source-specific 16S rRNA gene sequence targets that were detected out of 654, 721 and 593 specific targets for human, grazer and shorebird fecal sources, respectively.



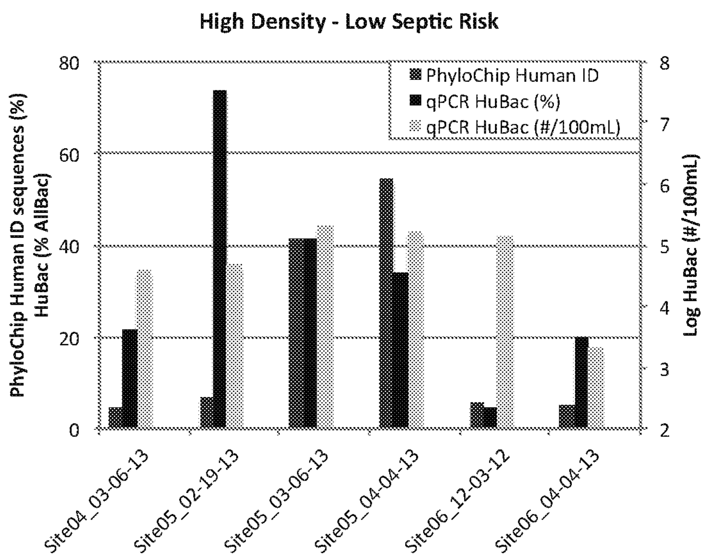
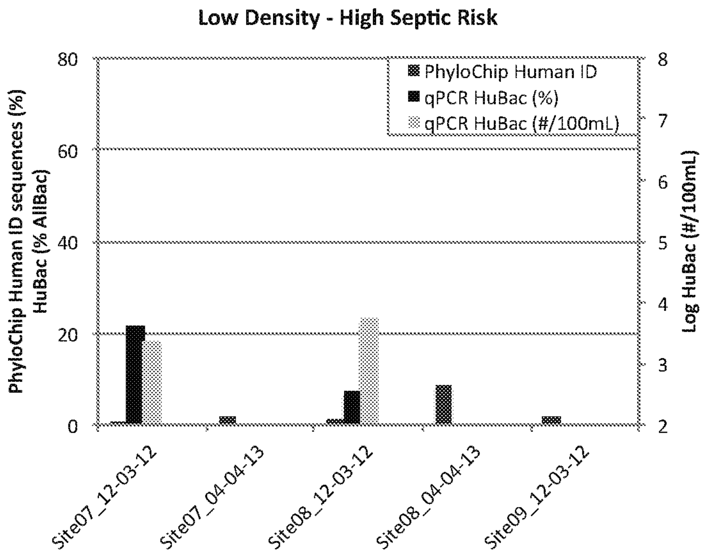


Figure 5-9. Comparison of PhyloChip human fecal ID results from high-density parcels to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.



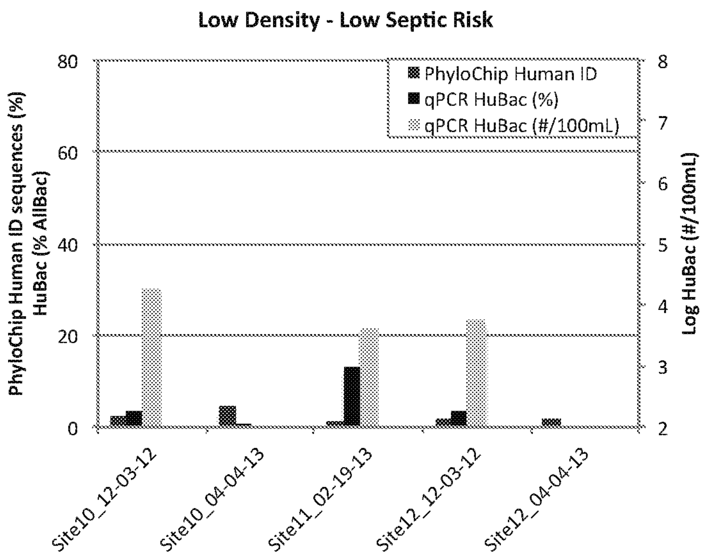


Figure 5-10. Comparison of PhyloChip human fecal ID results from low-density parcels to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.

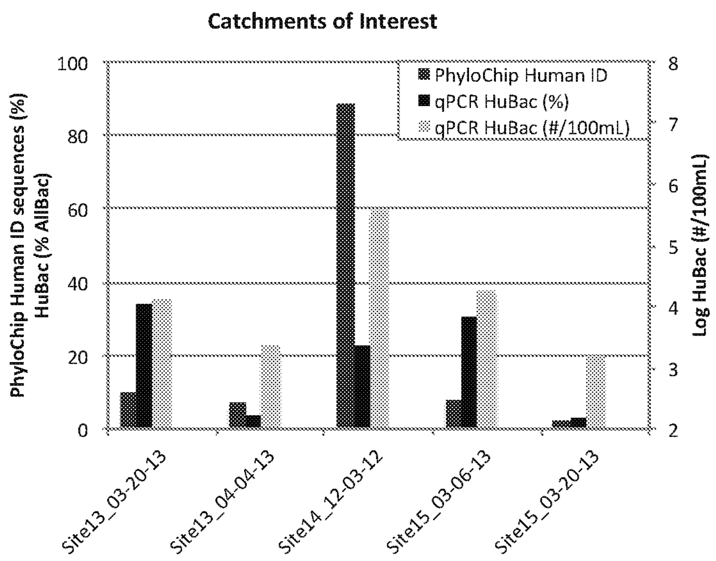
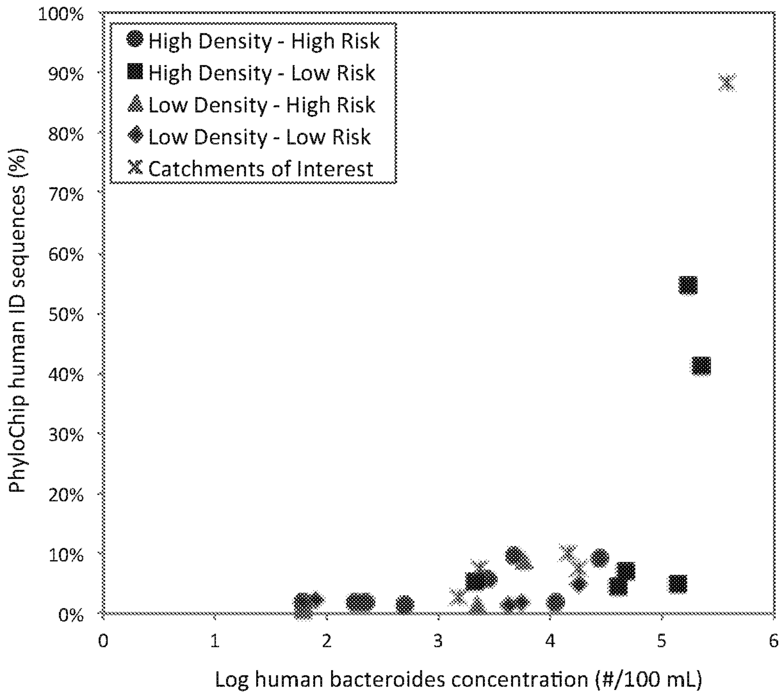


Figure 5-11. Comparison of PhyloChip human fecal ID results from catchments of interest to qPCR estimates of human *Bacteroides* measured by the HuBac test. PhyloChip results are reported as the percent of 16S rRNA gene sequences that were detected out of 654 human-specific sequences targeted by the test. HuBac results are reported as both estimates of concentration (#/100mL) and concentration relative to total *Bacteroides* measured by the AllBac qPCR test.



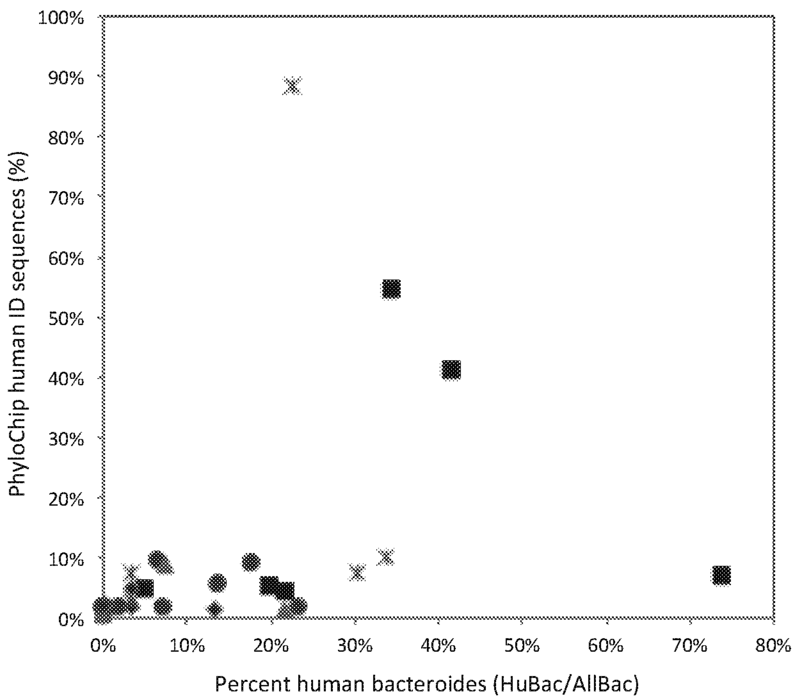


Figure 5-12. Relationship between PhyloChip human fecal ID results from Task 4 to qPCR estimates of human *Bacteroides* concentration (a) and human *Bacteroides* relative abundance (b). Correlation r=0.60 and r=0.37 for PhyloChip human ID % vs human *Bacteroides* concentration (a) and relative abundance (b), respectively.

APPENDIX A

Potential pathogens detected by PhyloChip in samples analyzed for Tasks 1 through 4. Values in red are number of detected OTUs that potentially include pathogenic strains. Counts of fecal indicator bacteria (yellow) and % diagnostic fecal source bacteria (blue) are included for comparison.

Task	Sample name	Station Name	Period	Land use	Total coliform (MPN/100mL)	E. coli (MPN/100mL)	Enterococcus (MPN/100mL)	Human ID (%)	Grazer ID (%)	Shorebird ID (%)	Klebsiella pneumoniae	Proteus mirabili	Salmonella enterica	Serratia marcescens	Shigella flexneri	Staphylococcus epidermidis	Staphylococcus haemolyticus	Streptococcus sp.	Vibrio cholerae	Yersinia sp.
Task 1	CSR.02	Commisky Station Road	Dry	NA	1789	10	52	1	3	3	0	0	0	0	0	0	0	0	0	1
Task 1	CRP.02	Cloverdale River Park	Dry	NA	3076	10	41	1	2	3	1	0	0	0	0	0	0	0	0	7
Task 1	GHB.02	Geyserville Hwy 128 Bridge	Dry	NA	1633	10	10	2	2	1	1	0	0	0	0	0	0	0	0	3
Task 1	AVC.01	Alex Valley Campground	Dry	NA	2625	10	31	2	2	3	1	1	0	0	0	0	0	0	0	0
Task 1	CR.01	Camp Rose	Dry	NA	2019	20	20	2	2	3	1	0	0	0	0	0	0	0	0	0
Task 1	MB.01	Memorial Beach	Dry	NA	3076	10	10	2	1	3	5	0	0	0	0	0	0	0	0	0
Task 1	SB.01	Steelhead Beach	Dry	NA	697	10	36	0	1	3	0	0	0	0	0	0	0	0	0	2
Task 1	FAB.01	Forestville Access Beach	Dry	NA	878	10	41	1	2	3	0	0	0	0	0	0	0	0	0	0
Task 1	JB.01	Johnson's Beach	Dry	NA	991	10	10	0	2	3	0	0	0	1	0	0	0	0	0	0
Task 1	MRB.01	Monte Rio Beach	Dry	NA	1439	10	10	1	2	2	0	0	0	0	0	0	0	0	0	0
Task 1	JBR.01	Jenner Boat Ramp	Dry	NA	6867	10	10	2	4	4	0	0	0	1	0	0	0	0	0	0
Task 1	DBC.01	Dutch Bill Creek	Dry	NA	341	10	10	5	6	10	39	0	1	3	0	3	0	1	0	0
Task 1	GVC.02	Green Valley Creek	Dry	NA	1086	31	187	1	5	8	1	1	0	1	0	0	0	0	0	0
Task 1	LSR.02	Laguna de Santa Rosa	Dry	NA	1500	74	52	1	3	7	1	0	0	0	0	0	0	0	0	0
Task 1	SRCL.02	Santa Rosa Ck at Los Alamos	Dry	NA	2845	192	379	5	6	7	31	0	0	3	0	0	0	0	0	0
Task 1	SRCR.02	Santa Rosa Ck at Railroad	Dry	NA	4611	246	97	1	4	4	0	0	0	1	0	0	0	0	0	0
Task 1	CSR.09	Commisky Station Road	Wet	NA	24196	767	959	0	1	2	2	0	1	0	0	0	0	0	0	2
Task 1	CRP.09	Cloverdale River Park	Wet	NA	4009	254	110	1	1	3	0	0	0	0	0	1	0	0	0	3
Task 1	GHB.09	Geyserville Hwy 128 Bridge	Wet	NA	3076	148	98	1	1	3	1	0	0	0	0	0	0	0	0	1
Task 1	AVC.09	Alex Valley Campground	Wet	NA	1259	74	20	1	3	3	0	0	0	0	0	0	0	0	0	0
Task 1	CR.09	Camp Rose	Wet	NA	1100	63	20	1	1	3	1	0	0	0	0	0	0	0	0	0
Task 1	MB.09	Memorial Beach	Wet	NA	4229	447	109	0	1	4	0	0	0	0	0	0	0	0	0	0
Task 1	SB.09	Steelhead Beach	Wet	NA	1248	36	26	2	12	5	1	0	0	0	0	0	0	0	0	0
Task 1	FAB.09	Forestville Access Beach	Wet	NA	1223	231	20	4	19	6	3	0	0	0	0	0	0	0	0	0
Task 1	JB.09	Johnson's Beach	Wet	NA	1071	63	10	54	17	4	5	0	0	0	0	56	5	0	0	0
Task 1	MRB.09	Monte Rio Beach	Wet	NA	1467	75	10	59	20	7	4	0	0	0	0	156	7	0	0	0
Task 1	JBR.09	Jenner Boat Ramp	Wet	NA	4478	121	20	16	3	3	0	0	0	0	0	0	0	0	0	0
Task 1	DBC.09	Dutch Bill Creek	Wet	NA	4352	10	31	1	3	6	4	0	0	1	0	0	0	0	0	0
Task 1	GVC.08	Green Valley Creek	Wet	NA	24196	1918	987	1	1	4	1	0	0	0	0	0	0	0	0	0
Task 1	LSR.08	Laguna de Santa Rosa	Wet	NA	24196	15531	5794	1	1	3	42	0	0	0	1	0	0	0	1	0
Task 1	SRCL.08	Santa Rosa Ck at Los Alamos	Wet	NA	10462	1455	504	1	1	2	0	0	0	0	0	0	0	0	0	0
Task 1	SRCR.08	Santa Rosa Ck at Railroad	Wet	NA	24196	2014	812	1	1	5	0	1	0	2	1	0	0	0	0	0
Task 2	AC.10	Abramson Creek	Dry	Ag	24196	1310	200	9	9	7	1	0	0	0	0	1	0	0	0	0
Task 2	LAC.14	Lambert Creek	Dry	Ag	24196	5830	1212	5	6	11	28	1	0	1	0	0	0	0	0	8
Task 2	WC.14	Woolsey Creek	Dry	Ag	24196	24196	24196	1	2	3	0	0	0	0	0	1	0	0	0	0
Task 2	MC.14	Mays Creek	Dry	Forest	24196	2341	1992	7	5	6	0	1	0	1	0	0	0	0	0	0
Task 2	PAC.10	Palmer Creek	Dry	Forest	308	5	22	6	8	12	29	0	0	2	0	1	0	0	0	0
Task 2	VBC.10	van Buren Creek	Dry	Forest	208	5	8	2	2	6	0	1	0	1	0	0	0	0	0	3
Task 2	IC.14	Irwin Creek	Dry	Septic	24196	17329	12997	1	2	6	0	0	0	1	0	0	0	0	0	1
Task 2	LIC.14	Limerick Creek	Dry	Septic	24196	6488	17329	52	11	8	20	1	0	0	0	0	0	0	0	0
Task 2	TC.10	Turner Creek	Dry	Septic	6668	515	98	9	15	9	0	0	0	2	0	0	0	0	0	0
Task 2	COC.10	Copeland Creek	Dry	Sewer	723	31	5	2	1	2	0	0	0	0	0	0	0	0	0	0
Task 2	FC.10	Foss Creek	Dry	Sewer	768	98	18	3	2	4	1	1	0	0	0	0	0	0	0	0
Task 2	PIC.10	Piner Creek	Dry	Sewer	1314	228	52	3	2	6	1	0	0	0	0	0	0	0	0	0
Task 2	BC.10	Blucher Creek	Dry	Shrub	1844	36	36	1	1	4	1	0	0	0	0	0	0	0	0	1
Task 2	CRC.14	Crane Creek	Dry	Shrub	24196	7969	6916	5	7	7	1	0	0	0	0	1	0	0	0	4
Task 2	GC.10	Gossage Creek	Dry	Shrub	1785	309	85	2	2	6	0	0	0	0	0	0	0	0	0	0
Task 2	AC.12	Abramson Creek	Wet	Ag	24196	24196	24196	16	36	14	0	0	0	0	0	8	0	0	0	0
Task 2	LAC.12	Lambert Creek	Wet	Ag	12691	20	31	6	5	4	34	0	0	1	0	0	0	0	0	0
Task 2	WC.12	Woolsey Creek	Wet	Ag	2116	8	10	12	18	10	0	0	0	4	1	22	0	0	0	0
Task 2	MC.13	Mays Creek	Wet	Forest	573	104	37	16	11	6	0	0	0	0	0	0	0	0	0	0
Task 2	PAC.11	Palmer Creek	Wet	Forest	4884	420	272	2	8	3	6	0	0	1	0	0	0	0	0	4
Task 2	VBC.12	van Buren Creek	Wet	Forest	24196	613	733	5	4	5	0	0	0	1	0	0	0	1	0	8
Task 2	IC.12	Irwin Creek	Wet	Septic	985	63	41	9	17	4	36	0	3	3	1	1	0	0	0	0
Task 2	LIC.12	Limerick Creek	Wet	Septic	19640	158	5	6	20	11	0	0	2	3	1	1	0	0	0	0
Task 2	TC.12	Turner Creek	Wet	Septic	24196	24196	24196	11	34	8	0	0	0	3	1	3	0	0	0	0
Task 2	COC.12	Copeland Creek	Wet	Sewer	24196	4352	9208	24	33	8	0	0	0	2	1	0	0	3	0	0
Task 2	FC.11	Foss Creek	Wet	Sewer	24196	6131	2046	16	3	6	0	0	0	1	0	0	0	0	0	0
Task 2	PIC.12	Piner Creek	Wet	Sewer	24196	5475	6488	32	13	19	0	0	3	0	1	0	0	3	0	0
Task 2	BC.12	Blucher Creek	Wet	Shrub	24196	12033	12033	12	33	6	0	0	4	1	0	1	0	0	0	0
Task 2	CRC.12	Crane Creek	Wet	Shrub	890	8	36	21	34	10	0	0	0	0	1	0	0	0	0	0
Task 2	GC.12	Gossage Creek	Wet	Shrub	24196	17329	24196	14	30	8	0	0	0	0	1	3	0	0	0	0

APPENDIX A

Potential pathogens detected by PhyloChip in samples analyzed for Tasks 1 through 4. Values in red are number of detected OTUs that potentially include pathogenic strains. Counts of fecal indicator bacteria (yellow) and % diagnostic fecal source bacteria (blue) are included for comparison.

Task	Sample name	Station Name	Period	Land use	Total coliform (MPN/100mL)	E. coli (MPN/100mL)	Enterococcus (MPN/100mL)	Human ID (%)	Grazer ID (%)	Shorebird ID (%)	Klebsiella pneumoniae	Proteus mirabili	Salmonella enterica	Serratia marcescens	Shigella flexneri	Staphylococcus epidermidis	Staphylococcus haemolyticus	Streptococcus sp.	Vibrio cholerae	Yersinia sp.
Task 3	JB.03	Johnson's Beach	Dry	NA	921	20	58	1	4	6	0	0	0	0	0	0	0	0	0	0
Task 3	JB.04	Johnson's Beach	Dry	NA	1046	16	30	0	2	2	0	0	0	0	0	0	0	0	0	0
Task 3	JB.05	Johnson's Beach	Dry	NA	980	24	37	1	3	3	0	0	0	0	0	1	0	0	0	0
Task 3	JB.06	Johnson's Beach	Dry	NA	816	7	30	14	2	2	0	0	0	0	0	1	0	0	0	0
Task 3	JB.07	Johnson's Beach	Dry	NA	1553	22	63	50	18	5	0	0	0	0	0	2	0	0	0	0
Task 3	MRB.03	Monte Rio Beach	Dry	NA	1733	3	11	2	3	2	0	0	0	1	0	0	0	0	0	0
Task 3	MRB.04	Monte Rio Beach	Dry	NA	1300	22	9	1	2	2	0	0	0	0	0	0	0	0	0	0
Task 3	MRB.05	Monte Rio Beach	Dry	NA	1986	22	18	1	1	2	0	0	0	0	0	0	0	0	0	0
Task 3	MRB.06	Monte Rio Beach	Dry	NA	1300	15	18	1	1	3	0	0	0	0	0	1	0	0	0	0
Task 3	MRB.07	Monte Rio Beach	Dry	NA	1300	22	21	0	1	2	0	0	0	0	0	0	0	0	0	0
Task 4	01_03-06-13	Site 01	Wet	Hi PD-Hi risk	6588	3179	220	6	3	4	0	0	0	3	0	0	0	0	0	0
Task 4	01_03-20-13	Site 01	Wet	Hi PD-Hi risk	1337	51	20	2	1	3	0	0	0	1	0	0	0	0	0	0
Task 4	02_03-20-13	Site 02	Wet	Hi PD-Hi risk	24196	152	24196	1	1	8	21	0	0	3	0	0	0	0	0	0
Task 4	02_04-04-13	Site 02	Wet	Hi PD-Hi risk	24196	187	5172	2	1	10	0	0	0	5	0	1	0	0	0	0
Task 4	02_12-03-12	Site 02	Wet	Hi PD-Hi risk	24196	1019	384	2	2	6	0	0	0	0	0	0	0	0	0	0
Task 4	03_03-06-13	Site 03	Wet	Hi PD-Hi risk	9804	160	432	2	1	3	6	0	0	0	0	0	0	0	0	0
Task 4	03_03-20-13	Site 03	Wet	Hi PD-Hi risk	24196	3654	216	9	2	4	26	[illegible]	0	2	[illegible]	0	0	0	0	0
Task 4	03_04-04-13	Site 03	Wet	Hi PD-Hi risk	12997	146	613	10	2	3	0	0	0	1	0	0	0	2	0	0
Task 4	03_12-03-12	Site 03	Wet	Hi PD-Hi risk	4106	158	295	2	2	4	0	0	0	0	0	0	0	0	0	0
Task 4	04_03-06-13	Site 04	Wet	Hi PD-Lo risk	24196	2613	12997	4	1	3	8	0	0	0	0	0	0	0	0	0
Task 4	05_02-19-13	Site 05	Wet	Hi PD-Lo risk	7933	393	86	7	2	4	17	0	0	2	0	0	0	0	0	0
Task 4	05_03-06-13	Site 05	Wet	Hi PD-Lo risk	24196	1664	3873	41	7	4	0	[illegible]	0	1	[illegible]	0	0	0	0	0
Task 4	05_04-04-13	Site 05	Wet	Hi PD-Lo risk	24196	4892	4950	54	14	9	0	0	0	2	[illegible]	0	0	0	0	0
Task 4	06_04-04-13	Site 06	Wet	Hi PD-Lo risk	24196	2755	24196	5	4	7	20	0	0	0	0	0	0	0	0	0
Task 4	06_12-03-12	Site 06	Wet	Hi PD-Lo risk	6488	246	211	6	2	4	8	0	0	0	0	0	0	0	0	0
Task 4	07_04-04-13	Site 07	Wet	Lo PD-Hi risk	1503	31	275	2	2	5	0	0	0	1	[illegible]	0	0	0	0	7
Task 4	07_12-03-12	Site 07	Wet	Lo PD-Hi risk	862	52	10	1	3	4	0	0	0	0	0	0	0	0	0	0
Task 4	08_04-04-13	Site 08	Wet	Lo PD-Hi risk	24196	1695	3551	9	3	9	0	0	6	0	[illegible]	0	0	0	0	0
Task 4	08_12-03-12	Site 08	Wet	Lo PD-Hi risk	9804	62	171	1	1	4	5	0	0	0	0	0	0	0	0	0
Task 4	09_12-03-12	Site 09	Wet	Lo PD-Hi risk	5172	327	85	2	2	3	0	0	0	2	0	0	0	0	0	0
Task 4	10_04-04-13	Site 10	Wet	Lo PD-Lo risk	24196	11199	7701	5	14	8	0	0	0	3	0	1	0	0	0	0
Task 4	10_12-03-12	Site 10	Wet	Lo PD-Lo risk	24196	323	410	2	2	7	0	0	0	0	0	0	0	0	0	0
Task 4	11_02-19-13	Site 11	Wet	Lo PD-Lo risk	8664	598	128	1	3	6	7	0	0	1	0	0	0	0	0	0
Task 4	12_04-04-13	Site 12	Wet	Lo PD-Lo risk	24196	121	2310	2	1	5	[illegible]	[illegible]	[illegible]	[illegible]	0	0	0	0	0	0
Task 4	12_12-03-12	Site 12	Wet	Lo PD-Lo risk	19863	171	139	2	3	5	7	0	0	0	0	0	0	0	0	0
Task 4	13_03-20-13	Site 13	Wet	COI	2142	122	98	10	6	4	0	0	0	4	0	0	0	2	0	0
Task 4	13_04-04-13	Site 13	Wet	COI	12997	3076	12997	7	7	8	0	0	2	0	0	0	0	0	0	0
Task 4	14_12-03-12	Site 14	Wet	COI	24196	2489	2481	[illegible]	23	10	47	0	0	1	0	0	0	1	0	0
Task 4	15_03-06-13	Site 15	Wet	COI	8164	31	41	8	2	5	4	0	0	0	0	0	0	0	0	0
Task 4	15_03-20-13	Site 15	Wet	COI	8664	238	605	3	2	7	0	0	1	3	[illegible]	3	0	1	0	0